The Functional Ecology of *Najas flexilis*

By
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Abstract

Najas flexilis (Willd.) Rostk. & Schmidt is a submerged rooted macrophyte. It is a Red Data Book species and is listed in Annexes II and IV of the EC Habitats Directive 92/43/EEC. It is also protected under domestic legislation, being listed on Schedule 8 of The Wildlife and Countryside Act, 1981, and Schedule 4 of The Conservation (Natural Habitats & c.) Regulations, 1994. In order to protect this species information is required on the ecological tolerances of the plant and the main threats to the species need to be elucidated. In addition, effective monitoring strategies are required to identify when the plant is endangered. The Biodiversity Action Plan for this species suggests reintroduction of *Najas flexilis* to extinct sites. If this is to occur information is required on when to reintroduce the plant, in what form (seed or plant) and where from. This thesis attempts to address some of these gaps in our knowledge of *Najas flexilis* ecology.

In this study, all Scottish post 1980 *Najas flexilis* sites were surveyed for the presence or absence of *Najas flexilis*. In addition, all pre 1980 mainland sites were visited and 10 Irish sites across the Irish geographic range and presence or absence of *Najas flexilis* recorded. The environmental conditions required for *Najas flexilis* growth, including water and sediment chemistry, light and exposure were evaluated. These environmental conditions are compared between present and extinct sites. Measurement of specific plant traits, leaf area and reproductive number, from a representative sample of the population is used as a method for assessing the condition of the population in response to the environment. Models show that the concentration of phosphate in the sediment and water and magnesium in the water, along with the alkalinity and either light or calcium in the sediment can predict 67 - 88% of the variation in the plant traits of *Najas flexilis*.

The community in which *Najas flexilis* grows was recorded from all sites visited. Two approaches were used a quantitative micro-habitat approach which is concerned only with the plants growing in the immediate vicinity of *Najas flexilis* and a qualitative whole lake approach. The suitability of these two approaches as a site condition monitoring tool for *Najas flexilis* is considered. The applicability of the Standing Water Type (SWT) classification to the community groups attained from whole lake approach and the applicability of National Vegetation Classification (NVC) scheme to the community groups attained from micro-habitat approach is considered. The lake scale qualitative approach elucidated significant differences in lake water pH, alkalinity and the concentration of total oxidised nitrogen and dissolved reactive phosphate in the water as

well as differences in phosphate, calcium and iron in the sediment. The small-scale quantitative approach only elucidated a difference in light extinction coefficient between community groups. The community groups attained from the small-scale quantitative survey were roughly comparable to NVC communities A13, A24a and A23. The lake scale qualitative approach produced community groups comparable to standing water type 3, 4 and 5, however, this study produced two type 5 sub-groups. Extinct *Najas flexilis* sites were classified as type 3 and the most eutrophic type 5, as were other sites that could still support *Najas flexilis*.

A generic trait based approach was applied to the question of the impact of competition on *Najas flexilis*. The quantity of a trait such as early flowering within the micro-habitat community was related to the success of the *Najas flexilis* population. The quantity of wind pollinated, June-July flowering perennials in the *Najas flexilis* micro-habitat can explain 68% of the variation seen in *Najas flexilis* leaf area/shoot length. These traits were then compared to those known to grow in similar habitats to *Najas flexilis* twelve native species possessed all of these traits. However, the invasive Elodea spp., which are thought to possibly cause a threat to Najas flexilis, do not possess all of the traits. *Elodea* spp. in Britain possess the perennial and June-July flowering traits, but are epihydrophilous. The importance of this is discussed.

Molecular techniques were applied to investigate the partitioning of genetic diversity of *Najas flexilis* in Scottish and Irish lakes. Life history traits and environmental factors were considered as reasons for the partitioning observed. RAPDs were used to study the genetic diversity of populations from ten Scottish, three Irish and one American site. All but two individuals could be identified as different genotypes. Individuals were generally more similar to other individuals within the same lake than individuals outwith the lake. No obvious geographic structure could be detected in the *Najas flexilis* populations. A significant relationship was not found between the water and sediment chemistry of the lake or the *Najas flexilis* trait data and the frequency of RAPD bands within a population. A direct relationship was found between the number of polymorphisms within a population and the exposure experienced by that population.

Najas flexilis germination response to various environmental stimuli was assessed experimentally. This is compared to the conditions in Scottish lakes. *Najas flexilis* germination is greatest in light deoxygenated conditions at warm temperatures (temperatures of 16°C is sufficient). If dark conditions prevail, germination is greater at

cooler temperatures (11°C). These conditions can be found in Scottish lochs in sheltered areas in June. Burial of seeds in lake sediment to a depth of 10cm did not appear to affect germination, although percentage germination was not as high as in the previous experiment at any depth.

This work contributes to the ecological knowledge of the species and provides information for the conservation of the species particularly with regard to threats to the species in Scotland and Ireland, monitoring for the species and reintroduction plans.

Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise acknowledged. It is entirely of my own composition and has not, in whole or part, been submitted for any other degree.

Ruth Wingfield
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1. Introduction

1.1 Introduction

Najas flexilis (Willd.) Rostk. & Schmidt is a submerged rooted macrophyte. It is listed in Annexes II and IV of the EC Habitats Directive 92/43/EEC. It is also protected under domestic legislation, being listed on Schedule 8 of The Wildlife and Countryside Act, 1981, and Schedule 4 of The Conservation (Natural Habitats & c.) Regulations, 1994. The Najadaceae have been classified as elodeid in form (Den Hartog & Segal, 1964). The vegetative structure of *Najas flexilis* appears visually similar to other members of this functional group, however it has some unusual attributes for an elodeid plant (or indeed any aquatic plant). *Najas flexilis* is relatively short in stature, rarely reaching above 30cm tall. It does not grow to the water surface and lives its entire life cycle completely submerged. The Najadaceae is one of nine plant families that flower underwater. Hydrophily (water pollination) occurs in less than 5% of aquatic species overall and is present in both marine and freshwater angiosperms (Cox 1988; Les 1988; Philbrick and Les 1996). What makes this state more unusual is that *Najas flexilis* cannot reproduce vegetatively. In this respect, it is unlike the majority of aquatic plant species, particularly those exhibiting hydrophily (Hutchinson, 1975). *Najas flexilis* is also an annual. This reliance on seed production, annual state and completely submerged life style is unusual for an aquatic plant and has consequences for growth, dispersal and establishment.

1.2 Description of *Najas flexilis*

Najas flexilis is a glabrous plant with linear, sessile leaves opposite or whorled, with denticulate sheaths. Flowers are inconspicuous, 1-3 are found in each leaf axil. This species is a monoecious annual, the flowers are hypogynous and sessile. The male flowers are surrounded by scales and possess one sessile anther; female flowers have one ovule, one carpel and 2-4 elongated stigmas, and are scaleless. The fruit is a sessile drupe (Hellquist & Crow, 1980; Preston & Croft, 1997; Stace, 1997). The seeds are easily identifiable by their smooth shiny brown coat. The seeds are elliptical, oblong, with a persistent membranous pericarp. The testa is smooth with squarish to hexagonal areoles regularly arranged in longitudinal rows (Triest, 1988) see Fig. 1.1.

1.3 Taxonomy

The Najadaceae family contains one genus, *Najas*, with forty species, all of which are submerged aquatics. Although the genus is cosmopolitan, the majority of the species can be

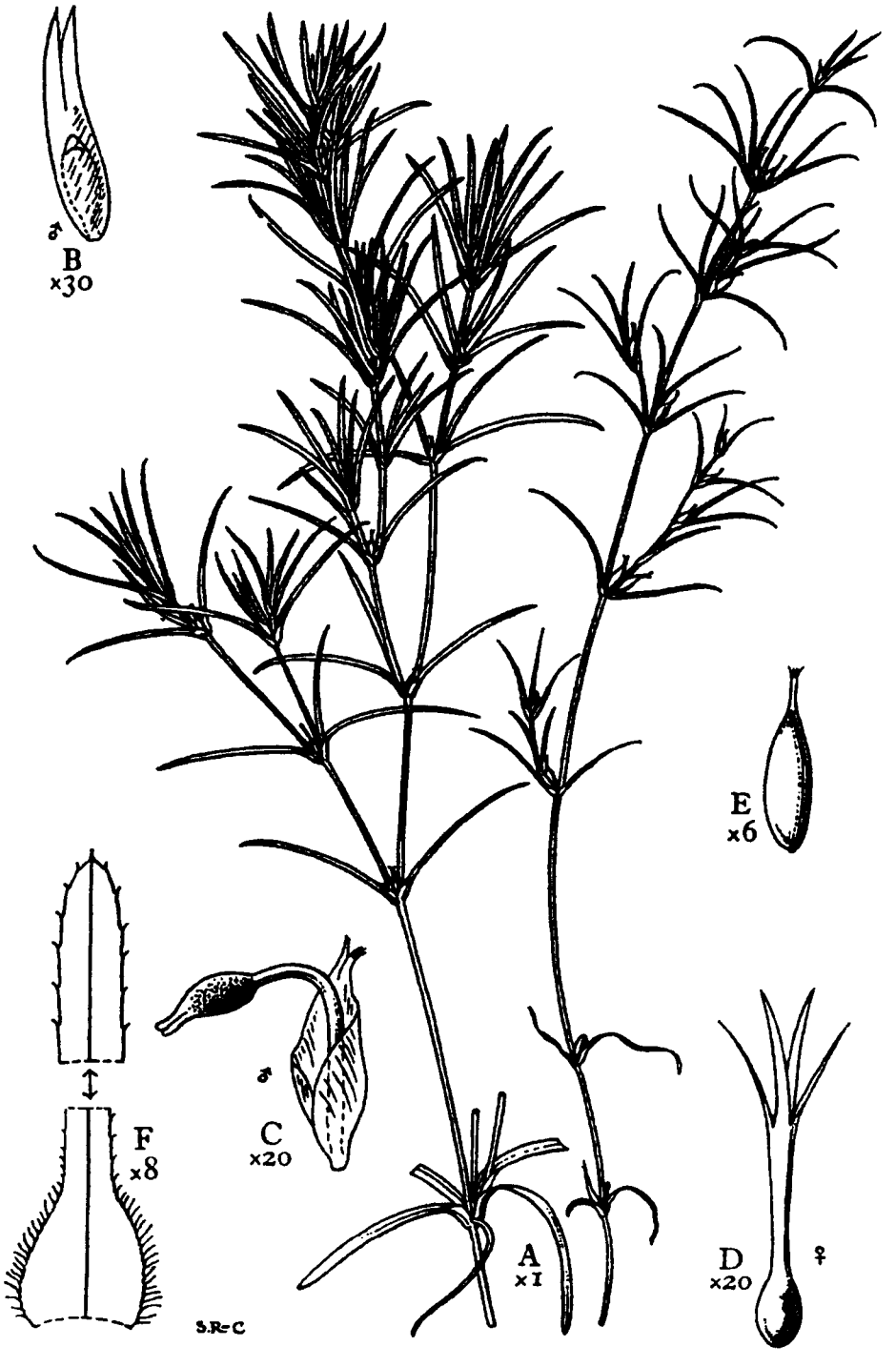


Fig. 1.1 *Najas flexilis,* A, flowering and fruiting stems; B, very young male flower, held within the sheath; C, male flower at dehiscence, after splitting of the sheath and elongation of the stalk; D, female flower; E, fruit; F, apex and lower part of a leaf. Plant bright deep green (Ross-Craig, 1979)

found in the tropical and subtropical regions (Cook, 1996; Preston & Croft, 1997). The species are either monoecious or dioecious and most are seed producing annuals. Vegetative reproduction in the entire genus appears to be rare (Triest, 1988). All the *Najas* species are hydrophilous and can grow in brackish or freshwater. Two subgenera are reported *Caulinia* and *Najas*. *Najas flexilis* belongs to *Caulinia*, which includes species that are usually slender, monoecious and lack spines on their stems (Triest, 1988).

1.4 Distribution

1.4.1 World and European

Najas flexilis can be found in North America, Europe and Asia. However, the frequency of its distribution is not even. The plant has a much denser frequency in North America (Haynes, 1979) compared to the sparse disjunct European range (Godwin, 1975). This distribution is not uncommon in aquatic plants. *Eriocaulon aquaticum* and *Potamogeton epihydrus* have an even more unequal cross-Atlantic distribution (Preston, 1995; Preston & Croft, 1997). The unequal distribution of *Najas flexilis* has been described by Hultén & Fries (1986) as amphi-atlantic. However, Preston & Hill (1997) describes the distribution as disjunctly circumpolar. The plant's range in Europe is not restricted to the west and its range in Asia is incompletely known, although it is found as far east as Mongolia (Hultén & Fries, 1986). This suggests that despite the denser frequency of sites in America the species is indeed circumpolar.

Within Eurasia, its climatic region is classified as Boreal-montane by Preston & Hill (1997). In western and central Europe, the Boreal-montane region is concentrated within the Alps, Scandinavia, Britain and Ireland. It is evenly spread across Britain, but concentrated in the north and west of Ireland. *Najas flexilis* has a northerly distribution in Europe today and can be found between the latitudes of 57° and 62° N. The British distribution of *Najas flexilis* is now limited to Scotland. Within Europe, the species is most densely frequent in Scotland and Ireland.

1.4.2 Scotland and Ireland

Najas flexilis was first found in Cregduff Lough just outside Roundstone in Connemara, Ireland in 1850 by Daniel Oliver (Oliver, 1851). It was later found in Scotland in 1875 at Loch Clunie, Perthshire (Sturrock, 1875). *Najas flexilis* was also found in England in 1914

by Pearsall (Pearsall, 1915). All records but one for *Najas flexilis* in England are from Esthwaite Water, the other is recorded from Lake Windermere (but the grid reference is for the Ferry House on the shore). Since these early records a total of 51 sites in Scotland have been reported to support *Najas flexilis* and 28 sites have been reported in Ireland (Table 1.1). The Irish sites are found predominantly on the west coast in three clusters one in the northwest in Donegal, one in Connemarra and one in Kerry. In Scotland, the distribution of *Najas flexilis* can again be grouped into three main clusters. The majority of the sites are found in the Outer Hebrides, on the west side of North and South Uist. Another cluster can be found in Kintyre and the Inner Hebridean Islands of Islay, Colonsay, Mull and Coll. The third cluster is in mainland Scotland in Perthshire. There are three further sites in mainland Scotland where the plant has been recorded, one in Dumfries and Galloway, one in Stirlingshire and one near Mallaig in northwest Scotland (Fig. 1.2).

1.4.3. Native?

When *Najas flexilis* was first discovered in Scotland in 1875 it was known that *Najas flexilis* was common in America and the status of *Najas flexilis* as a native species was questioned. The native status of *Najas flexilis* was particularly questionable as the Rev. Mr M. Ritchie, the minister of the parish of Cluny, and an enthusiastic botanist, was known to have introduced *Stratiotes* and *Butomus* to Clunie loch. However, after Mr Duff, the 'intelligent and obliging' gardener, confirmed that these plants had all been introduced from Wales (where *Najas flexilis* had not been found) it was considered by Abraham Sturrock that *Najas flexilis* was a native rarity of Loch Clunie (Sturrock, 1975). Sturrock went on to find a further five new locations for the plant in the Perthshire region in the next ten years.

Despite the increasing number of sites where *Najas flexilis* could be found in Scotland, the question of an American origin lingered due to its apparent amphi-atlantic distribution. Such questions were finally quashed by fossil evidence. Fossil evidence showed that *Najas flexilis* had been part of the British and Irish flora in the Hoxnian interglacial, the Ipswichian interglacial and then from the middle Weichselian through into the Flandrian (=Holocene) where it was both more widespread and more abundant than it is today (Godwin, 1975). Godwin (1975) states that:

'In a nutshell the present exiguous and extreme westerly range of *Najas flexilis* in this country is shewn by the fossil evidence to be the consequence of withdrawal in late Flandrian time from a much larger and more easterly range, and an occupation of very long

duration.' He also believed that *Najas flexilis* is a species of circumpolar distribution whose range has been reduced and restricted in Europe and Asia.

Fig. 1.2 The distribution of *Najas flexilis* in Britain and Ireland

Table 1.1 The sites where *Najas flexilis* has been recorded in the British Isles. (Six figure grid references are given if available).

Site	Grid reference	Date first recorded	Date last recorded	Date last surveyed
Mainland				
Nr River Tay, Aberfeldy	NN85-49-	1929	1929	1929
Loch a' Bhada Dharaich	NM695946	1977	2000	2000
Loch of Butterstone	NO062453	1986	1999	1999
Loch of Clunie	NO116444	1875	2000	2000
Loch of Craiglush	NO044446	1977	2000	2000
Fingask Loch	NO165431	1881	1999	1999
Loch Kindar	NX968640	1959	1996	1998
Lindores Loch	NO26-16-	1878	1878	2000
Loch of Lowes	NO055443	1883	2000	2000
Loch of Drumellie	NO14-44-	1881	1997	1999
Lake of Menteith	NN577002	1994	2000	2000
Monk Myre	NO20-42-	1883	1883	1999

Site	Grid reference	Date first recorded	Date last recorded	Date last surveyed
Loch Monzievaird	NN84-23-	1884	1884	2000
White Loch	NO16-42-	1881	1882	1999
Inner Isles & Kintyre				
Loch Ballyhaugh, Coll	NM176582	1987	1999	1999
Loch Fada, Colonsay	NR383955	1902	1999	1999
Glenastle Loch, Islay	NR294450	1994	1999	1999
Loch Gorm, Islay	NR230655	1993	2000	2000
Loch Lossit, Islay	NR408652	1949	2000	2000
Loch Nan Gad, Kintyre	NR787575	1971	2000	2000
Loch Poit na h-I, Mull	NM314227	1921	1999	1999
Loch Skerrols, Islay	NR34-63-	1949	1950	1950
Tangy Loch, Kintyre	NR695280	1973	1998	1998
Loch an t-Sagairt, Coll	NM251609	1989	1999	1999
Outer Isles, North & South Uist				
Loch Altabrug	NF745340	1984	2000	2000
Benbecula Aerodrome	NF79-56-	1950	1950	1950
Loch Bun an Ligidh	NF769371	1994	2000	2000
Loch Ceann a Bhaigh	NF76-30-	1940	1940	1940
Loch nan Capull	NF754161	1983	2000	2000
Loch nan Cnamh	NF762355	1994	1999	1999
Loch Cuilc	NF772369	1994	1998	1998
Loch na Cuithe Moire	NF738234	1983	2000	2000
Loch Druidibeag	NF780388	1987	2000	2000
Loch Dun Mhurchaidh	NF79-54-	1940	1940	1940
Loch Eaval	NF724710	1977	1999	1999
Loch an Eilean (Drimsdale)	NF762372	1984	1999	1999
Loch Eilean a' Ghille-ruaidh	NF769366	1984	1999	1999
Loch an Eilean (South Boisdale)	NF748169	1983	1999	1999
Loch nam Faoileann	NF752210	1995	2000	2000
Loch Gearraidh Mhic Iain	NF763360	1994	1999	1999
Loch Grogary	NF717710	1942	1999	1999
Loch Leodasay	NF80-63-	1982	1982	1982
Loch a'Mhadaidh	NF762353	1994	1999	1999
Loch a' Mhuilinn	NF74-33-	1947	1951	1951
Loch Olaidh Meadhanach	NF758314	1987	2000	2000
Loch Runavat	NF730693	1974	1974	1974
Loch Scarie	NF718705	1994	2000	2000
Loch an Taigh-sgoile (Schoolhouse)	NF763364	1984	1999	1999
Loch ? (no name)	NF766369	1994	2000	2000
Staoinebrig	NF74-33-	1939	1939	1939
South Uist, unnamed loch	NF737327	1994	1994	1994
England				
Esthwaite Water	SD360965	1914	1982	1994
Lake Windermere	SD390956	1977	1977	1977
Ireland				
Lough Adoolig	00645740	1985	1985	1985
Lough Akibbin	24068183	1977	1991	1991
Anaserd Lough	026--4--	1936	1936	1936
Ard Bay Lough (probably Nageeron)	0275-31-	1991	1991	1991
Barnahallia Lough	0259-55-	1975	1975	1975

Site	Grid reference	Date first recorded	Date last recorded	Date last surveyed
Carragh lough	00717905	1877	2000	2000
Cloonee Loughs	008--6--	1957	1957	1957
Clooney Lough	137--9--	1939	1939	1939
Cregduff Lough (Roundstone)	02718392	1850	2000	2000
Lough Dahybaan	13000200	1978	1978	1978
Fin Lough	02840660	1937	1937	2000
Fort Lake	2400-34-	1989	1989	1989
Glenade Lough	13828461	1978	1978	1978
Kiltooris Lough	1368-96-	1989	1989	1989
Lough Kindrum	25193429	1916	2000	2000
Kylemore Lough	02770583	1977	1977	1977
Leane Lough	00932890	1885	2000	2000
Lehanagh Lough	028--5--	1975	1975	1975
Mullaghderg Lough	14767203	1919	2000	2000
Lough Nafeakle	02683475	1977	1977	2000
Lough Nageeron	027--3--	1974	1974	1974
Lough Nagreary	2414-41-	1990	1990	1990
Lough Namanwaun	0266-39-	1881	1881	1881
Pollacapul Lough	02755620	1977	2000	2000
Renvyle Lough (Rusheenduff Lough)	02673640	1957	2000	2000
Sessiagh Lough	24040360	1981	1989	1989
Lough Shennagh	24213452	1989	2000	2000
Sheskinmore Lough	13700960	1981	1981	1981
Tully Lough	02692620	1978	1978	1978

This reduction in sites since the Flandrian time is possibly due to a decrease in temperature since the plant was at its most abundant at the time of the thermal maximum, in zone VI of the early/mid Flandrian. The requirement for long warm summers can be extrapolated from the fossil records in the Boreal times. At such times, *Najas flexilis* was found as far north as Lapland in Finland where it is no longer found today (Vasari, 1962). However, it can also withstand cold winter temperatures, as shown by its distribution today. An example of this is that *Najas flexilis* can be found on the Mongolian highlands where the summer temperatures are high but the winter temperatures are as low as -20°C. *Najas flexilis* would also have had to withstand more constantly cold temperatures when it grew in Britain in the Middle and Late Weichselian. However, Bell (1969) believes that even then the summer average temperatures would not have been so very cold, at approximately 16°C. However the middle and late Weichselian lasted tens of thousands of years and during this time the climate fluctuated greatly. The records of *Najas flexilis* from the cold stages of the Quaternary have recently been listed by West (2000).

As well as a reduction in temperature the other reason suggested for the restriction of the distribution of *Najas flexilis* since Boreal times is the lack of appropriate habitats. In particular, the number of calcareous or basic lake basins unaffected by drainage and other human interference such as eutrophication has been reduced (Godwin, 1975).

This lack of lakes unaffected by human interference is emphasized by the fact that not all the *Najas flexilis* sites that have been discovered still support *Najas flexilis*. Eight lakes in Britain and Ireland where *Najas flexilis* had previously been found were searched specifically for the plant in this study (and other recent surveys), yet the plant has not been found (Table 1.1). It is this decrease in site numbers that has led to concern over the status of this species in Britain and Ireland. However, it is not only in Britain where the species is thought to be in decline. *Najas flexilis* is also reported to be on the decline in Ohio possibly due to increased turbidity of the lakes (Wentz & Stuckey, 1971).

1.5 Habitat-macrophyte interactions

The distribution of *Najas flexilis* on the global scale, as controlled by climate and dispersal has been discussed. Within Scotland and Ireland, which is the primary focus of this study, the distribution and the decline of *Najas flexilis* will be controlled by habitat availability and this will have repercussions on dispersal (due to geographical distance between sites). The habitat will limit both whether *Najas flexilis* is present within a lake, and where it occurs within a lake. The factors that are responsible for limiting *Najas flexilis* growth, will be both biotic and abiotic. The same factors are likely to limit all macrophyte growth, but the range of these factors that *Najas flexilis* can withstand is unknown. One of the aims of this PhD study was to determine where *Najas flexilis* fits within the range of environmental conditions that support macrophytic growth.

The main factors that affect macrophytes and specifically *Najas flexilis* are illustrated in Fig. 1.3. The individual macrophyte will be influenced by other macrophytes and algal growth via competition, which will limit the resources available to the individual. As well as limiting resources through competition, macrophyte growth can also modify the environment, for example trapping sediment and slowing water movement. The individual is capable of competing with other individuals of the same species as well as individuals of other species. It is also capable of utilising and modifying the environment like any other macrophyte. Therefore, any factor, which can interact with the competing species, can also interact with *Najas flexilis*. This is why diagrammatically there is no gap between competitors and the

individual (Fig.1.3). Arrows pointing to or from the macrophyte and algae block includes the individual *Najas flexilis* plant block. The presence of other organisms within the water body such as zooplankton and detritivores obviously also directly or indirectly affect the ability of the individual macrophyte to grow. However, this is not an attempt to describe the food web, but the environmental interactions with macrophytes and shall therefore not be discussed here.

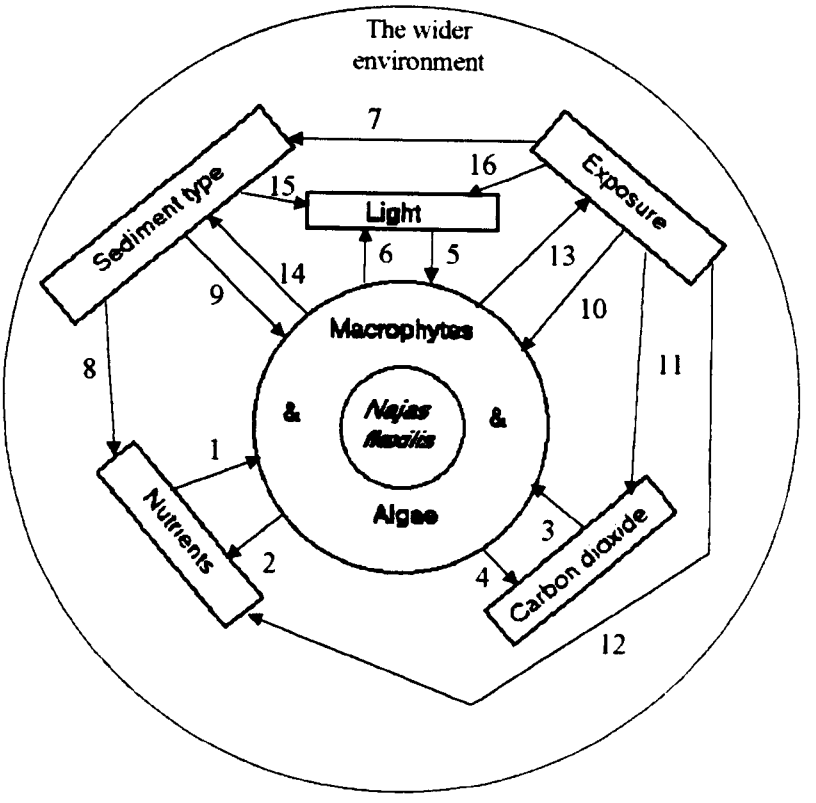


Fig. 1.3 Interactions between *Najas flexilis* and the environment. The numbers identify the interactions, details of the interactions are in the text (section 1.4.1-1.4.5).

1.5.1 Nutrients

Whether aquatic plant nutrients are obtained from the water or the sediment has often been debated e.g. Robach *et al.* (1995). However, Carignan & Kalff (1980) have shown that nutrients are most likely to be gained from the sediment for most rooted macrophytes, including *Najas flexilis*. The nutrients most often thought to be limiting for macrophyte growth is nitrogen (Best *et al.*, 1996) and phosphorus (Moeller *et al.*, 1988) although other

nutrients may also be limiting. Sculthorpe (1967) states that the ions of major metabolic significance in freshwaters are; potassium, calcium, magnesium, iron, ammonium, nitrate, sulphate, chloride, phosphate and bicarbonate. The lack of nutrients may be exacerbated by competition, although an abundance of these nutrients may make another resource, such as light or carbon dioxide, limiting due to an abundance of plant growth. Therefore a lack of the required nutrients will limit plant growth (interaction 1), and plant growth will both reduce nutrient supplies as they grow and replenish them when they die (interaction 2).

1.5.2 Carbon dioxide

In still waters where the demand for carbon dioxide for photosynthesis is high, carbon dioxide concentration can become limiting for plant growth. In such situations, bicarbonate can be utilised by some species (Hough & Fornwall, 1988; Jones *et al.*, 1996; Spence & Maberley, 1985; Spencer & Bowes, 1990). However, the cost of using bicarbonate is high and requires more energy in the form of light than is needed for CO_2 utilisation, another important limiting factor for macrophytes. It is suggested by Spencer & Bowes (1990) that plants with a minimal bicarbonate use are often slow growing, benthic plants limited to low pH waters, whereas those capable of high bicarbonate use such as *Myriophyllum spicatum* are more likely to cause weed problems. *Najas flexilis* has been classified as an obligate carbon dioxide utiliser. (Hough & Wetzel, 1978). Therefore, whilst the concentration of CO_2 limits aquatic plant growth (interaction 3) aquatic plant growth also reduces the CO_2 concentration as it photosynthesises and replenishes the supply as it respires (interaction 4) (Wetzel, 1983).

1.5.3 Light

Macrophyte growth is often limited by light regardless of carbon dioxide concentration. The quantity of light reaching a macrophyte depends on what it hits first, be that macrophyte or water. If it is water, it depends whether the surface is rough or smooth, and this is affected by exposure (Madsen *et al.*, 2001). If the water surface is rough less light reaches the aquatic plants due to light being reflected at the surface. The quantity of light reaching the macrophyte also depends on how much water the light has to travel through, i.e. the depth at which the plant grows (Middelboe & Markager, 1997). It also depends on the colour of the water and how many particles it has in it, this can be due to the catchment properties. In this situation light is lost due to absorption by suspended biotic and abiotic particles and

colloids (Kirk, 1994). This will alter with the turbidity of the water. Turbidity will depend on the sediment type and the exposure the sediment is exposed to (15 & 16). Whilst insufficient light will limit macrophyte growth (interaction 5) abundant macrophyte growth will shade other plants and reduce the quantity and quality of light available to them (interaction 6). Macrophyte growth may also reduce turbidity and therefore increase light availability (Moss, 1998); this is discussed in the next section. Even if sufficient light reaches the bottom of a lake, siltation, marl deposits and periphyton on the leaves of the plants can again prevent light reaching the plant itself.

1.5.4 Exposure and sediment type

Water movements, which are a result of exposure, affect the particle size of the sediments. Where water movements are great the fine nutrient rich substrates and the organic matter are eroded away (interaction 7). This generally leaves nutrient poor, low organic matter, coarse substrates (interaction 8) (Spence *et al.*, 1973). Due to nutrients being gained mostly from the sediment, this has a potential to constrain plant growth. The ability of plants to root in such coarse sediments is also a problem (interaction 9).

The stability of the sediment, which is a function of sediment type and exposure, has a direct effect on plant growth. Erosion of the sediment will result in the dislodgement of the macrophyte (Schutten & Davy, 2000) or loss of plant parts (Doyle, 2001; Hudon *et al.*, 2000; Schutten & Davy, 2000) (interaction 9 & 10). Whilst settling of sediment will initially bring in nutrients to the plants, it will eventually shade and then bury the plants. This will effect both the growth phase, the establishment phase and propagules, whether these are seeds, turions or tubers (Frankland *et al.*, 1987; Hartleb *et al.*, 1993; Moore *et al.*, 1993). However, a certain amount of exposure and erosion can be beneficial. Such erosion of the sediment and dislodgement of propagules and their consequent movement within the water body is potentially important for gene flow (Kirsten *et al.*, 1998). It has also been reported that *Myriophyllum spicatum* plants benefit from exposure as this reduces the amount of epiphytes that can grow on them (Strand & Weisner, 2001). A certain amount of exposure and water movement is also beneficial as it increases the flux of carbon dioxide and nutrients across the diffusive boundary layer (Madsen *et al.*, 2001) (interaction 11 &12), yet only slightly greater water movements can constrain growth due to mechanical stress. This fits with an intermediate disturbance hypothesis, suggesting that a certain amount of

disturbance is beneficial but too much is detrimental. However, the limits of disturbance that *Najas flexilis* can withstand are hitherto unknown.

Macrophytes can also reduce the effects of exposure (interaction 13). Macrophytes have been found to reduce wave energy and current velocity within macrophyte beds of the littoral zones of lakes (Losee & Wetzel, 1993). This leads to an increase in sedimentation (interaction 14) a decrease in resuspension and turbidity and consequently an increase in light (interaction 6).

Exposure is affected by lake size, shape, and orientation and is a product of the intensity and frequency of the winds creating the waves (the wider environment) (Keddy, 1982; Weisner, 1987). The exposure experienced by the plant will depend on the depth of water in which it occurs and the quantity of macrophytes growing there. As depth also has a direct relationship with the quantity of light reaching the plant, Spence (1982) suggested that plants lived in zones determined by these two environmental variables. Where the extent to which wave action or light determines zonation depends on the relative amount of the macrophyte zone lying within or below the wave mixed zone. He believed that where the plant occurred mainly within this zone sediment type and exposure would exert primary control of zonation, growth form and biomass of the vegetation. Outwith this zone primary control is via light quality and quantity. In contrast, Rørslett & Agami (1987) found that the distribution of *Isoetes lacustris* in a Norwegian fjord was not affected by the exposure gradients present, only the light gradient. These differences may be due to the fact that Rørslett & Agami (1987) studied one particular species which is relatively robust, within one fjord, whilst Spence investigated the response of vegetation as a whole in response to exposure and light in many lakes.

It is not only exposure that is affected by the wider environment, including climate, local geology and topography. All of the environmental factors considered here are affected by the properties of the catchment and man's impact upon it. This is why all the environmental factors are enclosed in a larger block in Fig. 1.3, which is the wider environment, which includes both the natural environment, the climate and anthropogenic impacts upon that environment.

1.5.5 Dispersal

If all these limiting factors are suitable for an individual's growth a plant has to first colonise a site. This will depend on dispersal ability, the environment within the water body and the wider environment. Water movements will affect dispersal within a water body. Outwith the water body it will depend on geographical distance and the presence of the required vectors to traverse this distance as well as the persistence of the diaspore.

1.5.6 Relevance to the study

The probable extinction of *Najas flexilis* from several Scottish sites may be due to one or several of these environmental factors in individual cases. A major aim of this study was to determine the relative importance of these factors in affecting *Najas flexilis* survival.

1.6 Conservation and *Najas flexilis*

The protection of *Najas flexilis* by the European Habitats Directive requires that Special Areas of Conservation (SAC's) are designated where the species grow, in order to protect it. The statutory body then undertakes surveillance of the species at these sites. Three such areas are proposed for *Najas flexilis*, one in Tayside, one in Coll and one in South Uist. These areas contain seventeen lochs reported to support *Najas flexilis*. In order that these protected sites are managed adequately, information is required on exactly what conditions are suitable for *Najas flexilis* growth, so that these conditions can be maintained. The European Habitats Directive requirements overlap with the targets and objectives for *Najas flexilis* according to the Biodiversity Action Plan (BAP) for this species. The three main objectives and targets of the BAP are to:

1. Clarify the status of the species in the UK.
2. Safeguard the remaining populations.
3. If feasible restore to former sites.

The majority of the proposed actions in the BAP (proposed actions 5.1.1, 5.2.1-5.2.3) relate to safeguarding the species via maintaining or creating suitable conditions for the plant to grow. However, at the advent of this study it was unknown precisely what these conditions were and what the threats to the species might be, although the restriction of light penetration due to weed growth or nutrient enrichment were suggested as factors causing loss or decline. Three further issues are also dealt with by the BAP proposed actions, these are to:

1. Establish a strategy for monitoring and maintaining the natural distribution of this species in Scotland (proposed action 5.1.2).
2. Collect seed from a range of sites for preservation at RBG Kew's seed bank (proposed action 5.3.1).
3. If *Najas flexilis* is found to be absent, but conditions are suitable, consider restoring to former sites (proposed action 5.3.2).

As the issue of seed collection is addressed as part of the work by the millennium seed bank, the three remaining primary conservation objectives for *Najas flexilis* to be addressed in this project can be summarised as:

- Determining the conditions in which *Najas flexilis* thrives and thereby also determining the main threats to the species.
- Determining how to monitor the species.
- Determining the best way to reintroduce the species to extinct sites.

1.7 Aims

This PhD study should provide information for the conservation objectives by fulfilling the following aims:

- To investigate the environmental conditions determining the distribution of *Najas flexilis* both within and between lakes.
- To investigate the ability of community descriptions to describe the lake environment where *Najas flexilis* grows.
- To investigate the effect of macrophyte competition on *Najas flexilis*.
- To investigate the population genetic structure of *Najas flexilis*.
- To investigate the environmental factors controlling *Najas flexilis* germination.

1.8 Structure of the thesis

Chapter 1 describes the taxonomy and distribution of the species. It provides an overview of a macrophytes interactions with the lake environment and the conservation objectives for the species. Chapter 1 also describes the aims of this PhD study.

Chapter 2 addresses the abiotic environment that *Najas flexilis* can tolerate by comparing extinct and present sites. It also suggests a method for determining individual plant health

and relates this to the abiotic environment to determine the effects of the environment on individual health.

Chapter 3 looks at whether plant communities can be used to indicate how good a site is for *Najas flexilis*. It looks at two different scales of macrophyte community classification, small scale quantitative sampling, classified using the National Vegetation Classification scheme (Rodwell, 1995) and lake scale qualitative sampling classified using Palmer's Standing Water Type classification scheme (Palmer, 1989). The two schemes are compared and their ability to distinguish between sites with different abiotic environments and sites, which can and cannot support *Najas flexilis*, is assessed.

Chapter 4 looks at whether invasive weeds are likely to be a threat to *Najas flexilis* and whether the traits they possess determines this. It also looks at which traits are most likely to cause a threat to *Najas flexilis*.

Chapter 5 looks at the genetic variability possessed by the Scottish and Irish populations of *Najas flexilis*. It explores the possible reasons for the observed partitioning of diversity, considering the biology of the species and the environment where it occurs. The results are discussed considering possible reintroduction.

Chapter 6 is the study of the germination ecology of *Najas flexilis* and the environmental cues it requires. This is important if reintroduction is to be from seed to ensure that introduced seed germinates.

Chapter 7 is the final discussion and synthesis of the findings of the study as a whole.

As *Najas flexilis* is protected under the Wildlife and Countryside Act, 1981 and the Conservation Regulations, 1994, all work was carried out under licence from Scottish Natural Heritage in Scotland and from National Parks and Wildlife in Ireland.

2. Fitness of *Najas flexilis* populations in relation to lake environmental conditions

2.1 Introduction

2.1.1 Aims

- Assess the current status of *Najas flexilis* in all post 1980 recorded sites in Scotland.
- Survey sites with older records where the plant is thought to be extinct, to determine the environmental habitat range of *Najas flexilis*.
- Determine the relationships between the target plant, and environmental factors, concentrating on plastic phenotypic traits of the species.
- Develop a predictive model of *Najas flexilis* success in relation to environmental controls.
- Use the model to assess what the threats to the present populations are.
- Determine the factors that control the distribution of the plant within lakes, so that *Najas flexilis* can be reintroduced to a position where it can thrive.

2.1.2 Background

Najas flexilis has its European stronghold in Scotland, with 35 sites at which there have been positive identifications during 1998-2000. There are also a number of sites where the plant is believed to be extinct. There are eight sites in Scotland and Ireland with previous records for the plant, where searches from 1998-2000 have failed to find it. *Najas flexilis* is also believed to be extinct at the only post 1980 site in England (Esthwaite Water) after a specific search for the plant in 1994 failed to locate it (Wade, 1994). Due to the high conservation status of the plant and this apparent trend of extinctions, knowledge of the environmental conditions controlling plant success is required in order to conserve it. An effective method of assessing plant success within a site is also required, so that at risk sites can be identified and appropriate action taken to prevent further extinctions.

2.1.3 Lake chemistry

Most of the information on the environmental conditions in which *Najas flexilis* grows is from American populations of the species. Studies on macrophyte community composition, in relation to environmental parameters in American lakes have elucidated a range of pH and alkalinity values at which *Najas flexilis* can grow (Table 2.1).

Table 2.1 The pH and conductivity range of sites where *Najas flexilis* can grow according to the literature on its distribution in North American lakes.

Environmental variable	Range	Reference
pH	6.0-9.0	(Jackson & Charles, 1998; Moyle, 1945; Roberts *et al.*, 1985)
Alkalinity (mg/l)	6-307.7	(Jackson & Charles, 1998; Moyle, 1945; Pip, 1988; Roberts *et al.*, 1985)

Table 2.1 shows that a circum-neutral pH appears to be important in the distribution of *Najas flexilis*. Experiments by Titus & Hoover (1991,1993) indicate that at a low pH the reproductive capacity of *Najas flexilis* is limited. They found that seed production per *Najas flexilis* plant dropped from 95.5 to 0.25 seeds/plant, when water pH was decreased from 7.5 to 5. *Najas flexilis* is an annual and therefore relies on seed for perennation, as a result at pH 5 *Najas flexilis* populations would quickly become extinct. It is not known where within the pH range of 7.5-5 seed production would begin to fall or whether in natural systems pH is the only factor controlling reproductive success.

Another example of *Najas flexilis* preferring a more neutral pH was shown after the liming of Thrush Lake in Minnesota. This was carried out to increase pH from 6.46 to above 7 in an attempt to improve fishing. The increased pH first lead to a decrease in *Nitella* spp., then *Najas flexilis* and *Potamogeton pusillus* appeared for the first time the following year. As conditions returned to their prelimed state, the *Nitella* spp. grew back, the competitive advantage of *Najas flexilis* was lost and it concurrently decreased (Hagley *et al.*, 1996).

Physiological experiments have also illustrated some of the relationships between *Najas flexilis* and its environment. Wetzel (1969) and Wetzel & McGregor (1968) show that calcium levels of up to 10mg/l and magnesium levels of up to 5 mg/l appear to have a positive effect on the rates of carbon fixation. However, calcium levels of >30mg/l and magnesium levels of >10 mg/l, both greatly exceeded in hard water lakes, lead to a decline in rates of carbon fixation and altered the secretion rates of dissolved organic matter in *Najas flexilis*. Increasing the level of sodium had the contrary effect of increasing both of the above. Wetzel & McGregor (1968) found that high concentrations of Ca^{2+} and Mg^{2+} resulting in low monovalent:divalent cation (M:D) ratios, inhibit carbon fixation, that Mg^{2+} can offset to a limited extent the inhibitory effects of Ca^{2+} and that M:D ratios >>1 provide

optimal conditions for growth. However, the mechanisms resulting in these effects were not explained.

Hough & Fornwall (1988) and Hough & Wetzel (1978) have illustrated that unlike hard water plants *Najas flexilis* is an obligate CO_2 user and cannot utilise HCO_3^-. This reduces the photosynthetic capacity of *Najas flexilis* in hard waters and therefore restricts its distribution. However, *Najas flexilis* has been reported to occur at pH 9.0 where there can be virtually no CO_2. This appears to be the upper limit of the plant's range and the plant has not been reported frequently at such a high pH (Table 2.1). All the pH measures from the studies referred to in Table 2.1 are snapshots of a lake's pH, and whilst *Najas flexilis* can obviously survive exposure to such a high pH for a short time, whether it can survive prolonged exposure to a pH as high as this must be considered.

Some of the isoetids have been reported to obtain carbon supplies from the soil via gas channels in the roots and stems. There are no reports in the literature of *Najas flexilis* possessing this capacity and the roots and stems of *Najas flexilis* are thin and brittle in comparison to those of the isoetids making the possession of such gas channels unlikely. Neither does *Najas flexilis* inhabit the truly oligotrophic waters that the isoetids do, where the utilisation of soil carbon supplies is so advantageous.

This might help explain the intriguing UK distribution of *Najas flexilis* (see section 1.3.2). In mainland Scotland it grows mostly in lakes along the Highland Boundary Fault, in the Hebrides it grows on the border of the division between machair and moorland and formerly on the border of hard Borrowdale volcanic rock and sedimentary Silurian rocks in the Lake District. These locations mean that almost without exception the lakes are influenced both by the base rich input of sedimentary rocks and sands and the water run off from the catchments of acidic peatlands or hard igneous rock, thus supplying the very particular conditions in which *Najas flexilis* appears to thrive.

The phosphorus requirement of the plant comes overwhelmingly from the sediment (Carignan and Kalff, 1987; Moeller *et al.*, 1988). This illustrates that for *Najas flexilis* the nutrient status of the sediment rather than the water is what is important for nutrient uptake. However, the nutrient status of the water is still important because it determines competition, especially from algae. Moeller *et al.*, (1988) also showed that *Najas flexilis* does not work as a pump for phosphorus to the water body as very little phosphorus is

released from the leaves to the water.

2.1.4 Physical factors; depth, light, exposure and sediment structure

Preston & Croft (1997) state that, in the British Isles, *Najas flexilis* can be found in water less than 1m deep, but is usually found at depths of 1.5 m or more. The depth at which macrophytes grow is a result of a number of factors: these include light and exposure. As depth increases the amount of light available to the plant decreases. This will be affected by the water clarity, which is altered due to suspended solids, algal growth and the properties of the catchment. *Najas flexilis* has been found by Pip and Simmons (1986) in Shoal Lake, Manitoba-Ontario at a depth of 12-14m. This is an extreme depth for any macrophyte and thought to be a result of exceptionally clear water, with Zen (zenith extinction coefficient) at 0.5-1% of the surface light, warm summer temperatures in the deep waters and the presence of oxygen in the sediments during the ice-free season.

Exposure affects the physical characteristics of the sediment, as well as having a direct effect on the plant. On exposed shores, elutriation takes place resulting in coarse particles and compacted soils, whereas on sheltered shores or at depth (where the effect of exposure is less) the finer soils develop (Spence, 1972). *Najas flexilis* appears to grow in a particular silty, organic, flocculent sediment. This has been noted by McKenna (1984) in Kahle Lake, US, and in Scotland, in the lakes in Perthshire by James and Barclay (1996) and as a general habitat requirement of the plant in the British Isles by Preston & Croft (1997). Pearsall (1920) also noted that in Esthwaite Water, English Lake District, *Najas flexilis* grows on fine semi-liquid muds with an organic content of 5-10 % and that it is the first colonist and takes the place often occupied elsewhere by *Nitella*. The intensity of exposure depends on the shape, depth and size of the loch as well as wind direction (Keddy, 1982; Weisner, 1987). Therefore, depending on the nature of the loch, suitable fine silty soils may have developed at a number of different depths. It is most probable that it will be an interaction between exposure and water clarity, which will primarily determine the locality of *Najas flexilis* within a lake.

2.1.5 Competition

Another factor determining the locality of *Najas flexilis* within a lake is interspecific competition. The competitive ability of *Najas flexilis* will vary depending on environmental conditions such as light and carbon dioxide concentration. It could be presumed that in hard

water lakes bicarbonate utilising species would have a competitive advantage over an obligate carbon dioxide utiliser such as *Najas flexilis.* However, bicarbonate utilisation requires more energy and Hough & Fornwall (1988) observed *Najas flexilis* to be significantly competitive when light limitation reduced the efficiency of bicarbonate user *Potamogeton pectinatus.*

It has already been discussed (section 2.1.3) how increasing pH from 6.46->7.0 increased the competitive ability of *Najas flexilis* over *Nitella* spp. However, it is also suggested that *Najas flexilis* thrived, in this situation, due to the disturbance during the liming of the lake and grew in trenches and holes presumed to be created by anchors and nets (Hagley *et al.*, 1996). This suggests that *Najas flexilis* is a disturbance tolerator, which might be expected due to its annual life cycle (Grime, 1988) and Pearsall's (1920) observation that it is often the first colonist. The plant's preference for the more liquid muds where there is often erosion or deposition of sediment, i.e. disturbance, agrees with this life strategy. Kahle Lake, US, is an artificial lake, which consists of an impounded creek. Two years after the lake's creation *Najas flexilis* was reported to be a problem species (Tazik *et al.*, 1982). This supports the view of *Najas flexilis* as an early colonist taking advantage of freshly disturbed environments. Further indication that disturbance is acceptable for *Najas flexilis* has been the failed attempts to control *Najas flexilis* growth by winter draw down in Kahle Lake, which is actually thought to have enhanced its growth (Tazik *et al.*, 1982). This pattern of events was also seen after the dredging of Lily Lake, Wisconsin, US. Previous to dredging *Najas flexilis* was not present in any of the studied areas, but the growth season after the disturbance *Najas flexilis* had replaced the less disturbance-tolerant species such as *Potamogeton robbinsii.* However, at one site within the lake it had already been out competed by the following growth season (Nichols, 1984). The decline of *Najas flexilis* after the cessation of disturbance suggests that *Najas flexilis* is more likely to be a poor competitor in stable conditions, and better suited to disturbed conditions, which could limit its distribution within lakes.

2.2 Methods

2.2.1 *Najas flexilis* presence and absence

During the course of the study, 42 lakes were investigated in Scotland, (July-September, 1998-2000), together with 10 lakes in Ireland (August 2000) see Table 3.1. A set of environmental factors likely to be relevant to the ecology of *Najas flexilis* in Scotland, was

included in a field survey of lochs from which *Najas flexilis* had been recorded (post 1980). Mainland sites where the plant had been recorded before 1980 were also surveyed, this included sites where the plant is thought to be extinct. Only mainland pre-1980 sites were surveyed in this study because of logistical reasons. At each site *Najas flexilis* presence or absence was recorded. Measurements of environmental factors could then be compared between sites where *Najas flexilis* was present or absent. Only sites where *Najas flexilis* had previously been recorded on at least one occasion were included.

The location of *Najas flexilis* populations was determined within a loch from past records, where available, especially the Scottish Loch Survey data (held by Scottish Natural Heritage) and the results of M. Gaywood's (personal communication) previous surveys for the plant. Other sources of information used were the surveys carried out on the lakes in Perthshire by James & Barclay (1996) and Howson *et al.* (1997) and the survey of South Uist lochs by staff of the Royal Botanic Garden, Edinburgh (McKean *et al.*, 1983). Three basic survey methods were used to locate *Najas flexilis* within the lake; boat, shoreline and snorkel surveys. Snorkel surveys were considered the most effective for finding *Najas flexilis*, agreeing with comments in the literature (Capers, 2000), and were adopted as the standard approach wherever feasible. Boats were used when the lakes were particularly large and there was no knowledge of exactly where the plant could be found; this enabled a larger area to be surveyed. When boats were not available, a shoreline search was carried out. A shoreline search included searching the drift, wading into the water as far as safety would allow, and throwing grapnels into the deeper water (Table 3.1).

In 1998 preliminary surveys were undertaken in a range of Scottish lakes to determine the best sampling regime. *Najas flexilis* was, in some smaller lakes, found in clear water in the shallows, where any sampling regime could be applied including abundance measures from quadrats. However in other lakes even in the relatively shallow water *Najas flexilis* individuals could not be seen to the water colouration, often highly peat stained especially in the lakes on the Inner and Outer Isles. *Najas flexilis* was also found at depths of up to 3m making observations difficult. Tests in clear water showed that values of abundance attained by grapnelling did not correspond to the abundance of *Najas flexilis* observed growing in the lake. *Najas flexilis* was consistently under recorded by this method, agreeing with comments in the literature (Capers, 2000). This was because the plants often broke and fell off the grapnel (*Najas flexilis* plants do not float and are very fragile). Alternatively due to the plant's slender form it would not become entangled in the grapnel. These preliminary

surveys showed that a realistic measure of plant abundance could not be gained at all sites where the plant grows. Scuba diving or the use of underwater video cameras could have been an option, but resources prevented the use of these two approaches.

2.2.2 Quantitatively assessing *Najas flexilis* status within a lake

It was important to see whether there was a relationship between the environmental factors, which appeared to differ between present and absent sites, and the success of *Najas flexilis* in sites where it still grew. A quantitative relationship between these parameters would give further evidence to indicate which environmental factors were causing the decline of *Najas flexilis* in Scotland. In order to determine the success of a plant at a particular site, plant abundance is often used. This was not possible for *Najas flexilis* due to a number of methodological reasons described above. Instead, measures of the plant attributes were used. In clear water, the plants required to gain these measures could be collected by duck diving or careful use of a grapnel, in this way entire plants could be dislodged from the sediment and scooped up. In low visibility lakes, populations of *Najas flexilis* could be located with the use of grapnels, and then the plants could be collected by use of an Ekman grab, which can collect entire plants for trait measurement.

2.2.2.1 Traits

Assessment of performance or success of a species involves the measurement of some relevant part of a plant to provide an index of growth rate or vigour. Typical measurements are leaf size, length and shape, plant height and flower or fruit characteristics, the latter providing information about reproductive allocation (Kent & Coker, 1995). Specific plant traits or combinations of traits are often used as indicators of a species' fitness. This has often been used to look at the genotypic effect on plant fitness (Dudash, 1990; Fischer & Matthies, 1998a; Fischer & Matthies, 1998b; Oostermeijer *et al.*, 1994; Oostermeijer *et al.*, 1995; Ouborg & Van Treuren, 1995; Ouborg *et al.*, 1991). Plant traits have also been used to compare the fitness of species within the same genus to different environmental regimes (Sultan, 2001). The ideal fitness measure would include; seed production, probability of seed germination, probability of seedling survival to adulthood and reproductive number of those individuals that survived to adulthood, as used by Dudash (1990). However complete experiments following the success of progeny are not always viable. Instead, probability of the seed reaching maturity and reproducing is substituted by reproductive weight or

vegetative success of the reproducing adult, as this represents the resources allocated or resources available to the offspring. Such measures are used by Sultan (2001).

Such fitness traits are phenotypically plastic within genotypic constraints, this plasticity will be determined by the way in which the genotype of the organism interacts with its environment. It is this environmentally driven plasticity which can be used to determine the realised niche of *Najas flexilis* in Scotland, as the patterns of fitness response (along with the growth traits that underlie them) determine the range of conditions in which species may survive and successfully reproduce and hence their ecological breadth (Sultan, 2001). Phenotypic plasticity has successfully been related to environmental variables in the field such as specific leaf area and depth as a function of light (Spence *et al.*, 1973).

Three whole plant samples were randomly collected from each population, and trait analysis undertaken on the fresh plants. The plant traits listed in Table 2.2 were measured for each plant. Leaf and root area was measured using a flatbed optical scanner downloading images to a PC. Customised Delta-T scan plant analysis software was then used to analyse the images to produce the required data. (Ali *et al.*, 1999). The problem with relating wild population plant traits to the environment is that they can be affected by the age of the individual, which is often unknown in wild perennial populations. However, this problem is avoided when measuring plant traits of an annual such as *Najas flexilis.* To account for the differences found in plants collected at the beginning, to those at the end of the field season all traits were divided by shoot length, a function of age.

Table 2.2 Traits measured for each *Najas flexilis* plant collected

Leaf area
Leaf weight
Root weight
Root length
Root area
Stem weight
Number of reproductive structures
Weight of reproductive structures
Shoot length
Number of branches

2.2.3 Environmental measures

In order to assess the relationship between *Najas flexilis* fitness and the environment a number of environmental measures were taken at each site. Conductivity and pH were measured with Hanna portable meters, and photosynthetically active radiation was measured with a Skye SKP210 twin-sensor meter, on site. The readings were taken at the water surface and 0.5m beneath the water surface. From these readings, the light extinction coefficient was calculated using the standard formula (Wetzel, 1983). The extinction of PAR was used rather than PAR itself in all the following analysis due to the methodological difficulties in measuring light in shallow waters. One water sample per lake was collected at a sub-sample of the sites (n = 35) and were analysed by the Scottish Environmental Protection Agency for alkalinity, iron, calcium, magnesium, ammonia, total oxidised nitrogen (TON), total phosphate (TP) and potassium. Dissolved reactive phosphate (DRP) was also measured this is the smallest fraction of orthophosphate and also the most biologically active it was also used in this study as it is used by Scottish Environmental Protection Agency for monitoring freshwaters. Therefore if this study could state the range of DRP concentrations that *Najas flexilis* could grow in this data could be compared to the data held by SEPA on Scottish lakes. All these measurements were made after filtration so exclude particulates. One sediment sample per lake was also taken where *Najas flexilis* was found to grow. These were collected by Ekman grab due to the deep water in which *Najas flexilis* grew. Therefore, all samples were collected from approximately the same depth in the sediment as the same apparatus was used at each site. The sediment was then dried in the oven at 80°C then ground with a pestle and mortar and sieved with a 2mm sieve to remove solid particulates. These were analysed by the Scottish Agricultural College for extractable phosphorus, calcium, and total iron and total nitrogen. A subset of sites (n=33) were also analysed for extractable potassium, sodium and magnesium content. These were measured in mg/kg or mg/l of dried sediment with particulates removed.

Exposure was calculated using a simplified version of the formula used by Keddy (1982) and Weisner (1987) where:

$$E=\sum_{i=1}^{8} \text{exceedance}_{45i^{\circ}} \times \text{fetch}_{45i}$$

Fetch is the distance in km from the nearest shoreline and E = exposure. In Keddy (1982) exceedance is the number of times the wind speed was greater than 12mph. Since a number of geographically disparate lochs were used in this study, exceedance could not be measured at every site, and the method was simplified. All west-facing compass points were

doubled, this is because the predominant wind direction for Scotland and Ireland is from the west. Therefore, exceedance was assumed to be 2 for westerly directions and 1 for the rest.

The method used does not take into account the differing wind force experienced by the different sites and assumes they are the same. This is obviously not true, some lochs occur right next to the Atlantic coast where wind forces are greater whilst others occur in sheltered hollows in the landscape. This method was used due to the geographic distance between sites preventing in depth meteorological measurements to be made. A better estimate of exceedance would make the calculation of exposure more accurate.

2.2.4 Competition

An Ekman grab was used to take 20 x 20 cm^2 samples of the vegetation, three such samples were taken within the *Najas flexilis* population community. The mean dry weight for the three samples was used as the quantitative measure of biomass, for that site. Roots were included in these samples, as nearly all roots were collected within the Ekman grab. In contrast to the measurements of the *Najas flexilis* plants it did not matter if the roots were detatched from the rest of the plant when the plants were extracted from the sediment collected in the Ekman grab in the laboratory, as only a measure of biomass was required. This was calculated twice; once including *Najas flexilis* plants and once excluding them. This measure was used as an indication of the total competition and the interspecific competition that the *Najas flexilis* specimen plants were experiencing from other macrophytes.

2.2.5 Data analysis

The sites where *Najas flexilis* was absent (i.e. not found in this study) are split into two groups. The first absent group contains the sites that appear to be the more oligotrophic absent sites and the second absent group contains the sites that appear to be the more eutrophic absent sites. The environmental variables of both the absent groups were compared to those where *Najas flexilis* was present, using a Mann-Whitney U test. This test was chosen due to the small sample size in the more oligotrophic absent group, and the non-parametric nature of the data due to the detection limit imposed on many of the environmental variables.

All data were tested for normality using Ryan Joiner test procedures and appropriate

transformations applied to normalise individual data sets. Multiple regression procedures were used to relate *Najas flexilis* fitness to environmental variables and biomass, residuals were tested for normality and randomness against fitted values on the completion of any modelling process. One-way analysis of variance using Tukey's post hoc test was used to determine the differences between the depth at which *Najas flexilis* was found under different light and exposure regimes.

2.3 Results

2.3.1 *Najas flexilis* presence and absence

The Mann-Whitney U tests show that there is a significant difference between the more oligotrophic absent group and the present group for pH, conductivity, alkalinity, calcium and potassium in the water and total iron in the sediment. There is a significant difference between the more eutrophic absent group and the present group for pH, alkalinity, calcium, magnesium and total phosphate in the water and available phosphate in the sediment (Table 2.3). This shows that it is only between the more eutrophic absent group and present group that phosphate is significantly different. It is also of note that water clarity was not significantly different between present and absent sites. Although algal growth was noted at some of the more eutrophic absent sites such as Loch Monzievaird, others such as White Loch were dominated by charophytes and had clear water, whilst present sites could have highly peat stained water, decreasing water clarity.

Table 2.3 The range of environmental variables measured at *Najas flexilis* present and absent sites, a) in the sediment, b) in the water. A * denotes a significant difference between the present and absent group with the *, $p \leq 0.05$, using a Mann-Whitney U test. For explanations of abbreviations see section 2.2.3.

a)

	Oligotrophic absent	**Present**	**Eutrophic absent**
Available P (mg/l)	0.9 - 2.9 (n = 2)	0.3 - 17.3 (n = 41)	5.3 - 72.9* (n = 7)
Median	1.9	2.6	21.8
Available K (mg/l)	--	4 - 166 (n = 31)	28.3 - 89.2 (n = 4)
Median	--	35.1	77.1
Extractable Mg (mg/l)	--	16.3 - 388 (n = 31)	54.1 - 189 (n = 4)
Median	--	87	146
Extractable Ca (mg/l)	145 - 5250 (n = 2)	198 - 331000 (n = 41)	805 - 25000 (n = 7)
Median	2697.5	950	3140
Total Fe (mg/kg)	27600 - 20600* (n = 2)	94.4 - 168000 (n = 41)	123 - 14500 (n = 7)
Median	24100	1250	216
Total N (mg/kg)	320 - 6220 (n = 2)	240 - 142000 (n = 41)	600 - 11400 (n = 7)
Median	3270	3320	1540
Extractable Na (mg/l)	--	8.3 - 300 (n = 29)	20.5 - 72.5 (n = 4)
Median	--	52.4	50.05

b)

	Oligotrophic absent	Present	Eutrophic absent
pH	6.46 - 6.98* (n = 2)	6.62 - 8.3 (n = 42)	7.45 - 9.01* (n = 7)
Median	6.72	7.46	8.64
Conductivity (us/cm)	54.9 - 116* (n = 2)	55 - 447 (n = 42)	158 - 410 (n = 7)
Median	85.45	235	215
Light extinction coefficient	1.35 - 4.4 (n = 2)	0.53 - 7.32 (n = 42)	0.91 - 5.18 (n = 7)
Median	2.88	1.91	1.83
Alkalinity (mg/l)	4.91 - 5.7* (n = 2)	6.71 - 69.71 (n = 29)	41.5 - 98.66* (n = 3)
Median	5.3	23.45	72.65
Ca (mg/l)	1.32 - 2.02* (n = 2)	2.06 - 33.4 (n = 30)	14.22 - 24.72* (n = 3)
Median	1.67	9.59	21.7
Fe (mg/l)	0.2 - 0.16 (n = 2)	<0.03 - 1.69 (n = 30)	0.04 - 0.35 (n = 3)
Median	0.18	0.21	0.18
Mg (mg/l)	1.54 - 2.12 (n = 2)	1.34 - 8.8 (n = 30)	6.4 - 12* (n = 3)
Median	1.83	4.8	8.8
N-NH3 (mg/l)	< 0.04 (n = 2)	<0.04 - 0.1 (n = 33)	<0.04 - 0.28 (n = 4)
Median	<0.04	<0.04	0.07
N-TON (mg/l)	<0.1 (n = 2)	<0.1 - 1.369 (n = 33)	<0.1 - 2.646 (n = 4)
Median	<0.1	<0.1	<0.1
P-DRP (PO4) (mg/l)	<0.003 (n = 2)	<0.003 - 0.045 (n = 33)	<0.003 - 0.074 (n = 4)
Median	<0.003	<0.003	0.012
P-TP (mg/l)	0.012 (n = 2)	<0.003 - 0.056 (n = 30)	0.014 - 0.16* (n = 3)
Median	0.012	0.0095	0.017
K (mg/l)	0.3 - 0.54* (n = 2)	0.16 - 2.82 (n = 33)	0.3 - 2 (n = 4)
Median	0.42	1.2	1.4

2.3.2 Quantitative assessment of *Najas flexilis* status within lakes

2.3.2.1 Traits

The plant fitness traits used in the model were leaf area/shoot length and number of reproductive structures/shoot length. Leaf area and leaf weight appeared to explain the same variation in fitness, however the leaf area measurements had a more normal distribution, so were chosen for use in the model. Root measurements were not used as the roots of *Najas flexilis* are very thin and brittle and may well have broken in collection, therefore there was not enough confidence in the root data for it to be used. Reproductive number was used instead of reproductive weight, as it was not affected by the maturity of these structures, which could be a problem as plants were collected throughout the *Najas flexilis* growth season. The variation in number of branches was shown by the leaf area/shoot length measurement, so was not included.

In unfavourable conditions for *Najas flexilis* growth, the two elements of fitness used, reproductive number and leaf area did not reduce uniformly together. In more oligotrophic lakes, leaf area was of an intermediate level, however the number of reproductive structures reduced dramatically. At one site only one seed was found in three mature plants. This agrees with the results of the experiments by Titus & Hoover (1993) that low pH reduces reproductive fitness in *Najas flexilis*. In other environmentally similar lochs, only one or two plants were found, so they could not be sampled for fitness. It is probable that this low frequency of plants and low seed frequency among plants is related. At the more eutrophic end of the range, plants appeared to have longer internodes, this reduced the total leaf area of the plants as there were less leaf nodes and therefore less leaves per shoot length. Shoot length was not any longer in these plants with extended internodes the site with the lowest leaf area/shoot length had a shoot length which ranged from 5.5-9.5cm (from 3 plants), where as the range of shoot lengths recorded from all plants measured at all sites was 2-28.5cm with an average of 13.5cm. The lack of nodes also reduced the number of reproductive structures as these develop in the leaf nodes. Both of these conditions could possibly lead to population collapse. This is illustrated in Fig. 2.1 and 2.2, where each fitness component is regressed against pH. Both of the plots are noisy as pH is not the only factor controlling fitness, but an indication of trophic state, however they illustrate the unequal decline in the two components of plant fitness. As a result of this situation (reproductive number/shoot length) x (leaf area/shoot length) is used as the measure of fitness to assess *Najas flexilis* status in the lakes surveyed (Fig. 2.3). It is also the measure

predicted by the resulting models. The number of reproductive structures describes the reproductive success of the adult plant and the leaf area indicates the resources available for allocation to the reproductive structures, which indicates the survival chances of that seed becoming a mature plant.

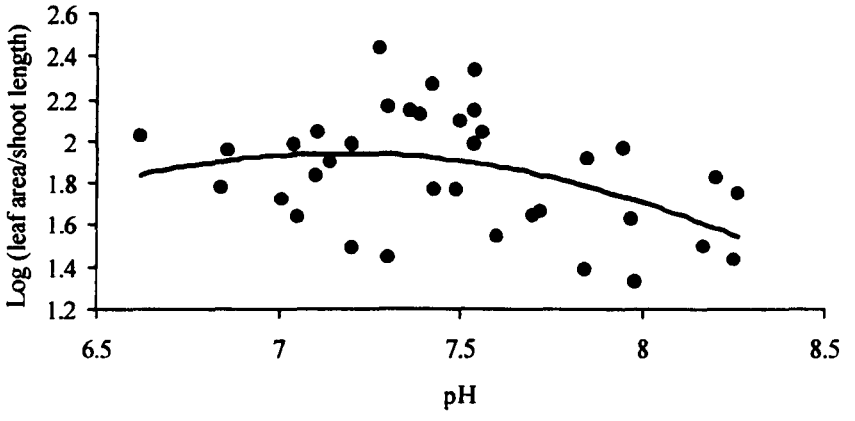


Fig. 2.1 The effect of pH on Log (leaf area/shoot length) $r^2 = 0.181$, $p < 0.05$

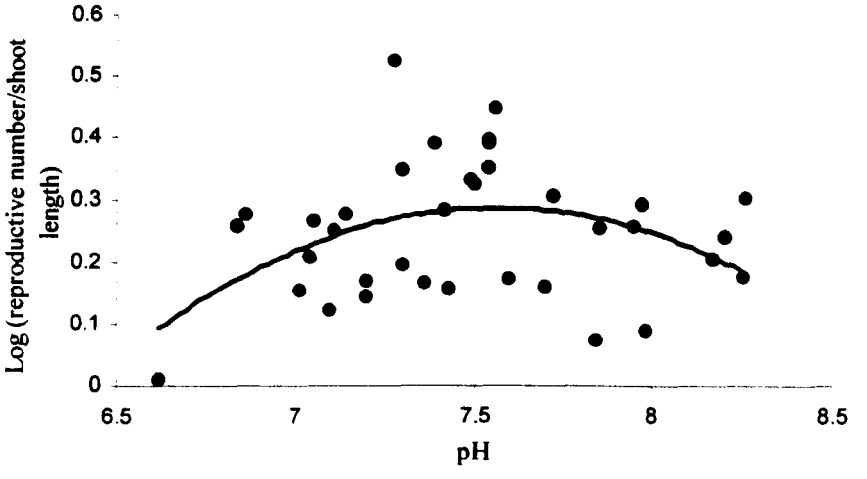


Fig. 2.2 The effect of pH on Log (reproductive number/shoot length) $r^2 = 0.165$, $p = 0.052$

Fig. 2.3 shows the log fitness for each site surveyed where three entire plants could be collected. Three sites have a log fitness value below one, these sites are those perceived to be at risk. It was also observed that at all three at risk sites *Najas flexilis* did not grow in as great an abundance as at other sites where the plants could be seen. Fig. 2.3 shows that there is a step increase between the three at risk sites and the rest of the *Najas flexilis* populations. Cregduff Lough despite having relatively leafy plants only had one seed between three plants. This suggests that the population size will be smaller next year, this site had the lowest pH value of all sites surveyed. Loch nan Cnamh and Loch an Eilean, South Boisdale, had a pH of 7.84 and 7.98 and possessed an average of 3 and 2 seeds per

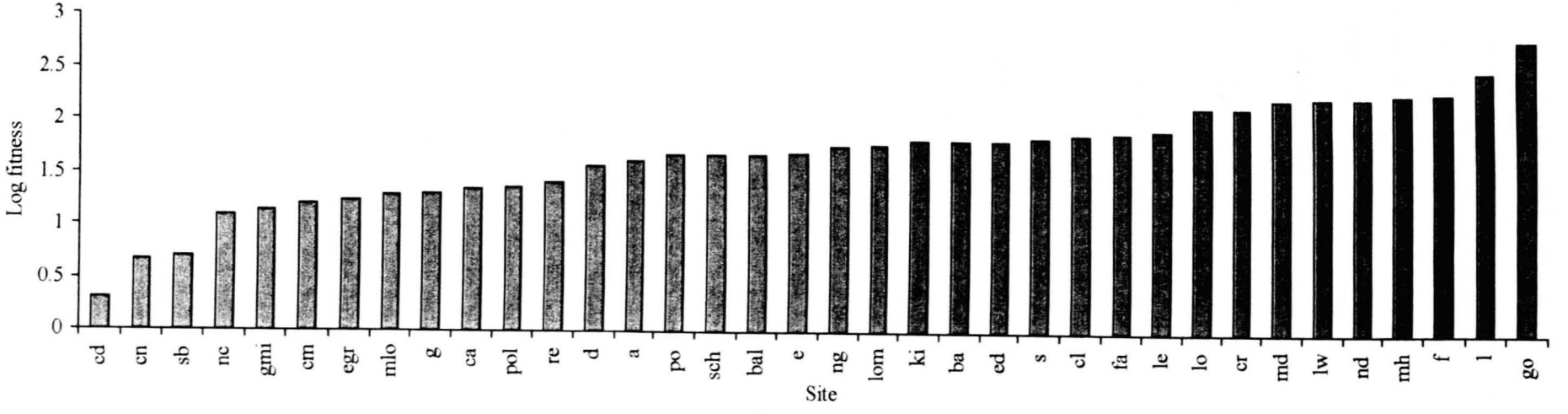


Fig. 2.3 The observed log fitness (leaf area/shoot length x reproductive number/shoot length) at all the sites where sufficient material could be collected. Site codes and names are in Appendix 1.

plant respectively, both sites also possessed plants with low leaf area/shoot length measures suggesting lower levels of resource to be allocated to seeds. Loch nan Cnamh was the site with the highest biomass and *Najas flexilis* appeared to be struggling to survive amongst the great amount of *Myriophyllum alterniflorum* and *Chara* sp. In contrast *Najas flexilis* in Loch an Eilean, South Boisdale had no competitors in the immediate vicinity, but grew in highly peat stained water, although the water was not of the lowest clarity out of the lakes visited. It can be assumed that both of these sites were suffering from a lack light due to either water coloration or macrophyte competition.

2.3.2.2 Predicting *Najas flexilis* fitness

The models for leaf area/shoot length and reproductive number/shoot length are intrinsically similar; this could be expected as one provides the resources for the other. Light extinction coefficient is a good indicator for predicting photosynthetic success as leaf area/shoot length. However it is not so good for predicting the number of reproductive structures produced, instead the quantity of calcium in the sediment is a better indicator. This is probably because it will affect the pH of the water, which appears to affect reproductive success.

Model 1: $n = 17$, $r^2 = 0.886$ $p < 0.005$

logLa/sl = 0.515 + (1.276logAlk) -(1.012logPs) + (50.775TPw) - (0.0921Mgw) - (0.599logLec)

Model 2: $n = 17$, $r^2 = 0.672$ $p < 0.005$

Rep/sl = 0.993 + (2.154logAlk) -(1.179logPs) + (44.801TPw) - (0.132Mgw) - (0.856logCas)

Model 3: $n = 17$, $r^2 = 0.768$ $p < 0.005$

logFit = -0.628 + (2.263logAlk) - (1.810logPs) + (84.067TPw) - (0.156Mgw) - (0.919logLec)

Model 4: $n = 17$, $r^2 = 0.743$ $p < 0.005$

logFit = 0.642 + (2.738logAlk) - (1.392logPs) + (59.509TPw) - (0.199Mgw) - (0.649logCas)

Where:

La/sl = Leaf area/shoot length

Fit = (Leaf area/shoot length) x (Reproductive structure number/shoot length)

Rep/sl = Number of reproductive structures/shoot length

Alk = Alkalinity mg/l

Ps = Extractable phosphorus in the sediment mg/l

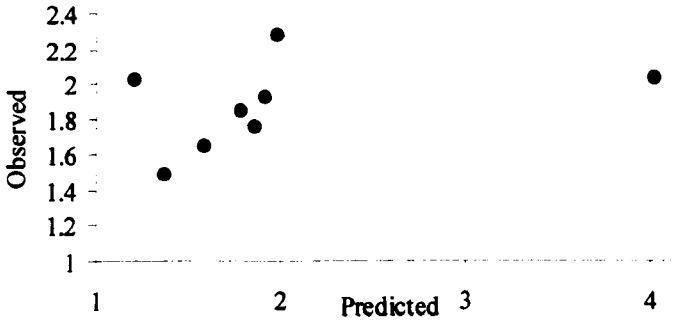
TPw = Total phosphate in the water mg/l

Mgw = Magnesium in the water mg/l

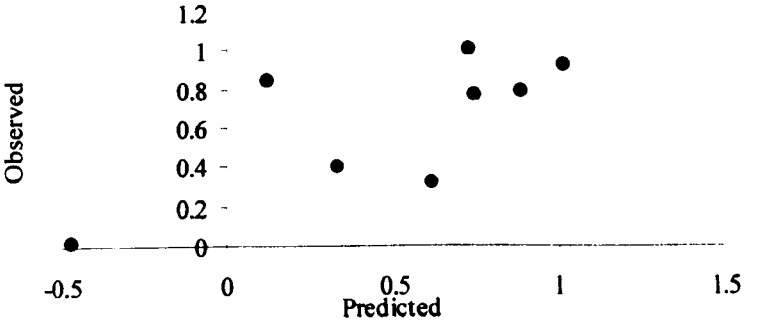
Cas = Calcium in the sediment mg/l

Lec = Light extinction coefficient

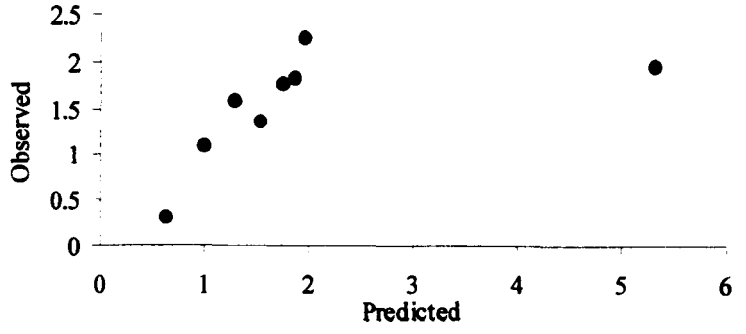
a)



b)



c)



d)

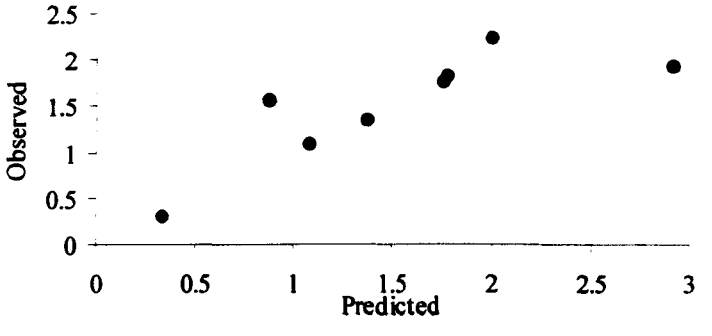


Fig. 2.4 The observed and predicted fitness values of *Najas flexilis* for 4 Irish sites and 4 additional Scottish sites, 3 in S. Uist and 1 in Kintyre, a) = model 1, b) = model 2, c) = model 3, d) = model 4. The site with a constantly higher prediction of fitness than that observed is Lough Leane

The models created were tested on the four Irish sites, where sufficient plant material was obtained, and three further Scottish sites from Kintyre and South Uist. The predicted values were plotted against the observed values (Fig. 2.4). The model predicting reproductive success alone, works the least well. All the other models appear to predict the fitness of *Najas flexilis* relatively well. The major exception from the test data is from Lough Leane where the fitness of the plant is consistently predicted to be greater than the observed fitness. Lough Leane is presently becoming increasingly eutrophic. This recent change may well be affecting the plants, but it is possible that phosphate will not yet have accumulated in the sediment, hence the over prediction of plant fitness. These results show that, with the exception of Lough Leane, the models can equally be applied to the Irish and Scottish *Najas flexilis* populations.

2.3.3 Competition

Macrophyte biomass (both including and excluding *Najas flexilis*) did not have a significant relationship with *Najas flexilis* fitness on its own. This is because there are many other factors also affecting the fitness of *Najas flexilis*. It did not contribute any further to the model either, as the nutrient levels in the water and sediment would be an indication of both algal and macrophyte growth as would the variables that indicate CO_2 concentration. Therefore although competition probably is playing a part in determining *Najas flexilis* fitness the data set is too noisy to determine a significant relationship, and other parameters in the model are more effective indicators of *Najas flexilis* fitness.

2.3.4 Depth

Fig. 2.5 shows that for the populations studied depth had a significant effect on the exposure level that could be endured by the *Najas flexilis* population. Only when exposure is low were *Najas flexilis* plants found in shallow water. Therefore, the depth of water in which *Najas flexilis* grows depends on exposure at that site. This does not show whether the depth at which *Najas flexilis* grows is due to exposure damaging the fragile *Najas flexilis* plants or whether it is due to the effect exposure has on the sediment texture.

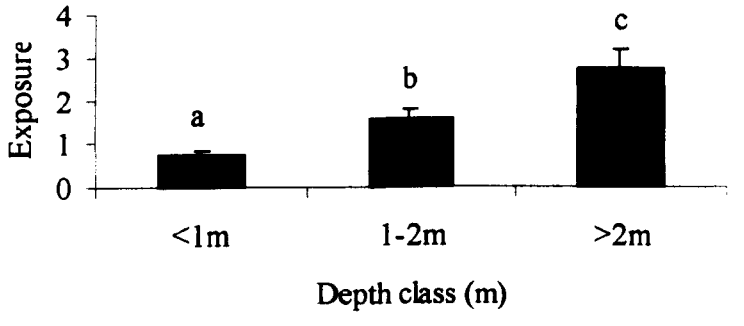


Fig. 2.5 The depth at which lake populations of *Najas flexilis* are situated in relation to surface exposure measurements. Different letters signify significantly different exposure values $p \leq 0.05$ using Tukey post hoc test after a one way analysis of variance.

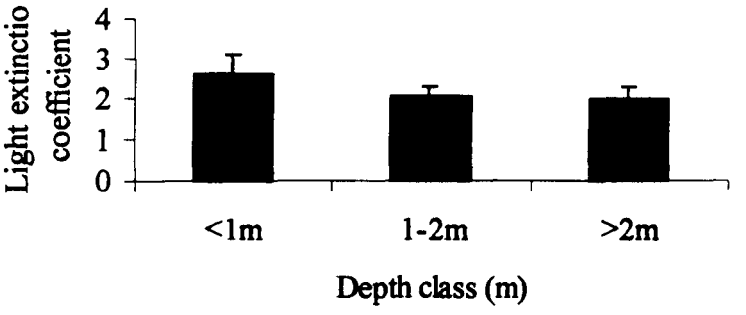


Fig. 2.6 The depth at which lake populations of *Najas flexilis* are situated in relation to light extinction coefficient measurements. No significant difference was found between the depth classes using a one-way analysis of variance.

Although there is no significant difference in water clarity between depth classes, Fig. 2.6 shows that there is a trend for sites with lower water clarity to have *Najas flexilis* populations in shallower water. This lack of a significant difference is most probably due to the sampling method, the first population of *Najas flexilis* encountered was usually the population surveyed and consequently this would be one of the shallowest populations. Whilst it will be the depth of the shallowest population that will be affected by exposure, it will be the depth of the deepest population that will be affected by light. This is because whilst plants in lakes with low water clarity can only grow in the shallows, in lakes with clear water and low exposure plants can grow from the shallows to the depths, thus merging the distinction between the three groups. The recording of plants at their maximum depth could have solved this. Therefore, Fig. 2.5 and Fig 2.6 only suggest that it will be an interaction between exposure and water clarity, which will determine the locality of *Najas flexilis* within a lake.

2.4 Discussion

There is an overlap between the ranges of the chemical parameters of the present and absent site groups. This is because the population at the absent site could have become extinct at any time since the last record, in some cases a period of over 100 years. Since the time of extinction, the lake quality may have improved to a state where *Najas flexilis* could theoretically grow, but recolonisation may have been hindered due to geographic distance from neighbouring populations or ineffective dispersal from these populations may have limited its re-establishment. Another possible reason why the ranges for the present and absent site groups are not discrete is that the environmental parameters do not affect *Najas*

flexilis independently and extinctions will be brought about by a number of contributing factors.

The water chemistry measurements are a snapshot of the lake water chemistry. The nutrient levels in the samples are low, as they are taken in summer, when most of the nutrients will be incorporated in the growing organisms. However, it is within this time period that *Najas flexilis* grows, so this is when the measurements were taken. As a result many of the DRP, TON, and NH_3 levels are below the detection limit. Therefore, no significant difference was found between presence and absence groups and their effect on the fitness of *Najas flexilis* could not be determined. This does not mean that the presence and absence groups do not have different quantities of these nutrients, or that they have no effect, just that it cannot be detected.

The more oligotrophic absent sites have significantly lower pH, conductivity, alkalinity, calcium and potassium levels in the water than the present sites. These low levels appear to affect the reproductive success of the plants, but not their photosynthetic capacity. Unless the plant is preferentially allocating resources to the photosynthetic tissue instead of the reproductive structures, which is an unlikely strategy for an annual plant, this suggests that these environmental conditions are inhibiting reproductive success. However, experiments on floral development and pollen viability in various environmental conditions are required to confirm this. These sites also have significantly higher iron sediment levels. The significance of this is unclear; iron is thought to be a micronutrient for aquatic plants (Sculthorpe, 1967). It is also linked to the oxygen status of the sediment and this in turn is related to whether or not phosphate is bound in the sediment. Both Fe II and phosphate can be released from the sediment in deoxygenated conditions (Hutchinson, 1975). However the sediment in *Najas flexilis* lakes was usually a grey colour rather than an anoxic black.

In contrast, the more eutrophic absent group has significantly higher pH, alkalinity, calcium, magnesium and total phosphate levels in the water and significantly higher phosphate levels in the sediment. These parameters do not fluctuate independently and are all integrally linked. The quantities of these parameters found within a lake will be due to both the nutrients and minerals available from the catchment and the interactions within the lake.

Alkalinity, pH and calcium levels in the water all indicate the concentration of carbon

dioxide in the water. Higher levels of these measures indicate that the main form of inorganic carbon in the water is bicarbonate. The pH, alkalinity and calcium levels and also the inorganic carbon equilibrium will be determined, in part by, the characteristics of the catchment, however they will also shift in accordance with the biological activity within the lake. High levels of photosynthesis by either algae or macrophytes within a lake will shift the equilibrium towards a bicarbonate system at least in the daytime. This is because photosynthesis will use up the available CO_2. Plants characteristic of such hard water systems avoid this problem by utilising HCO_3^-, however this requires significantly more energy than CO_2 utilisation. *Najas flexilis* is an obligate CO_2 user and can not utilise HCO_3^- (Hough & Fornwall, 1988). However it can still compete in marginal habitats by living in low light conditions, usually at greater depths, where there is not enough light to fuel bicarbonate utilisation, it is also in these depths where any available CO_2 may be found.

High levels of photosynthesis require high nutrient levels as well as light. As nitrogen and phosphorus often limit primary production in mesotrophic systems (where *Najas flexilis* grows) an increase in these nutrients would increase photosynthetic production, which would decrease available CO_2 concentrations. This would seriously limit the growth of *Najas flexilis* as in such an environment it would be incapable of fixing carbon. This would result in a situation where plants would produce less leaves, leaf nodes and therefore less reproductive structures, and this is seen in lakes with a high pH (which indicates low CO_2 levels), see Fig. 2.1. If this situation continued, less seeds would be produced each year and the population size would decline. The significantly higher levels of phosphate in the water and the sediment and the higher pH, alkalinity and calcium levels, in the more eutrophic absent sites, suggest that this may be the cause of extinction. The absent eutrophic sites in this study are all found in the more intensive agricultural areas in Perthshire, suggesting phosphate sources from agriculture may be a threat to the species. Other potential sources of phosphate, referred to in the *Najas flexilis* BAP plan, is from fish farms although at present, sites containing fish farms successfully support *Najas flexilis*.

Whether the increase of photosynthesis would be by macrophytes or periphyton and phytoplankton was not determined, although both would have a similar effect. The abundance of periphyton and phytoplankton was not measured, but periphyton was observed growing on *Najas flexilis* at some sites despite reports that *Najas flexilis* releases phosphate only sparingly to epiphytes and water (approximately 2% of cumulative phosphate uptake after five weeks) (Moeller, *et al.*, 1988). Phytoplankton was also

observed in sufficient abundance to restrict plant observations when snorkelling in some, but not all of the more eutrophic sites.

Models 1 and 3 show that light is also important for predicting plant success. The quantity of photosynthetically active radiation reaching *Najas flexilis* plants will decrease as algal and macrophyte growth increases, as these will intercept the light before it reaches the relatively low growing *Najas flexilis*. *Najas flexilis* like all plants is limited in its distribution by light availability. However, it naturally grows at low light levels and can grow productively in unclear water, this is shown by the low light extinction coefficients in lochs supporting *Najas flexilis*. This suggests that photosynthetically active radiation would have to be severely limited to cause *Najas flexilis* extinction. The fact that some of the absent *Najas flexilis* lochs, dominated by charophytes, have exceedingly clear water now does not mean that they always have. Charophyte populations often boom and bust and with these cycles water clarity also improves and declines and *Najas flexilis* could have become extinct at that site during a decrease in water clarity.

2.5 Conclusions

The fitness measures developed here can be used to monitor and assess the present *Najas flexilis* populations and elucidate which populations are at risk. The models can be used to calculate the predicted fitness of *Najas flexilis* at absent sites to determine if the lake quality is suitable for reintroduction of *Najas flexilis*. Where there are no records of where *Najas flexilis* previously grew within an absent lake, the relationship between depth and exposure can be used to determine where within a lake *Najas flexilis* should be introduced to, in order that it can thrive. The models and analysis of present and absent sites have also suggested the major threats to the species in Scotland to be those related to eutrophication and acidification.

Summary

- *Najas flexilis* present and eutrophic absent sites differ significantly in pH, alkalinity, calcium, magnesium and total phosphate levels in the water and phosphate levels in the sediment.
- *Najas flexilis* present and oligotrophic absent sites differ significantly in pH, conductivity, alkalinity, calcium and potassium levels in the water and total iron in the sediment.

- *Najas flexilis* fitness can be assessed using the trait measurements (leaf area/shoot length) x (reproductive number/shoot length)
- *Najas flexilis* fitness in relation to the lake environmental conditions can be predicted with reasonable success for Scottish and Irish sites.
- The depth at which *Najas flexilis* grows is significantly affected by exposure
- There is a trend between the light extinction coefficient and the depth at which *Najas flexilis* grows, this may be significant if improved methodologies were used.

3. Aquatic plant community description at different scales; approaches to describe the environment in which *Najas flexilis* grows

3.1 Introduction

3.1.1 Aims

- Determine whether plant community descriptions are a suitable tool for assessing site condition.
- Compare and assess two approaches to macrophyte community classification; the whole lake qualitative approach and the micro-habitat quantitative approach for their ability to differentiate different freshwater environments.
- Assess the present British classification schemes for their applicability for this purpose.
- Assess the suitability of these schemes for assessing site condition for *Najas flexilis.*

3.1.2 Background

Najas flexilis is listed in Annex 2 of the European Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (The 'Habitats Directive'). This requires member states to identify sites for this species, which will be designated as Special Areas of Conservation (SACs). A number of *Najas flexilis* sites in Scotland have already been put forward as SACs. SACs as well as Special Protection Areas (SPAs) and Special Sites of Scientific Interest (SSSIs) must undergo 'site condition monitoring' (SCM) which means that 'interest features' (e.g. *Najas flexilis*) on these sites must be monitored once every six years. The results for SACs and SPAs (European designations) will be passed onto Brussels, as required under the Habitats Directive. The Habitats Directive does not state how the monitoring should be done, that is to be decided by the organisations concerned.

Common standards for SCM are being developed by the UK nature conservation agencies. One common standard agreed on is nature conservation objectives for each interest feature (e.g. *Najas flexilis*) on each site, which define when the condition of an interest feature is favourable. Such objectives should include characteristics (attributes) for assessment of the interest feature against set targets. Targets can directly describe the interest feature, or be good indicators of the condition of the interest feature. The degree of acceptable change of the characters should be described and the characters should be comparable across all sites where the interest feature is found.

Submerged aquatic plants are intrinsically difficult to survey as they often grow in low-visibility water. Not only is locating the plant problematic, but determining the plants condition once its been found is also difficult. This is because obtaining measures of plant success such as abundance is not easy if the plant cannot be seen. Destructive techniques are also not ideal as *Najas flexilis* is a protected species under the Wildlife and Countryside Act, 1981 and the Conservation Regulations, 1994, and therefore any destructive surveys require a licence. One solution is SCUBA diving, but this is expensive for the nature conservation agencies to carry out. As a consequence the use of indicators of the condition of the interest feature, i.e. *Najas flexilis* is appealing. Using plant community descriptions as one such indicator is presently being considered by English Nature.

A qualitative plant community description is much easier to obtain than a quantitative survey of plant abundance within a water body. If only qualitative information is required, non-quantitative survey methods, such as grapnelling, can be used; this reduces both the cost and time involved. However, it is important to know how good an indicator such descriptions would be, and how variable they can be, before the interest feature (*Najas flexilis*) is also impacted. There have been several classifications of British aquatic plant communities, it is important to determine whether these community descriptions could be applied as indicators to the monitoring of *Najas flexilis,* or whether new community descriptions, solely for this purpose, are required.

3.1.2 Aquatic plant communities in Britain

Aquatic plant communities of British lakes have been classified by Pearsall (1920), Spence (1964), Palmer (1989), and Rodwell (1995). Some have been limited in their geographic area, Pearsall concentrated on the Lake District and Spence on Scotland, excluding the Western Isles. West (1905, 1910) also concentrated on Scottish lochs. These classification systems do not only differ in the geographical regions they consider, but also the scale at which they look at aquatic plant communities.

3.1.3 Survey methods used for deriving plant community descriptions

Pearsall (1920), Spence (1964) and Rodwell (1995), are all concerned with classifying community types within aquatic systems, i.e. there will usually be more than one community

type per lake. For this purpose, Spence (1964) and Rodwell (1995) took a number of quadrats to devise their respective communities. For the UK National Vegetation Classification (NVC), the sizes of these quadrats were $2x2m^2$ or $4x4\ m^2$ (Rodwell, 1995), Spence (1964) does not clarify the quadrat size used. Pearsall (1920) noted the presence and relative abundance of plants as they were brought up on the hooks of a dredger. Regardless of the exact area surveyed, all of these classifications are concerned with individual communities within lakes, as well as among them. The different communities these authors find within a single lake are most often assigned to different depths, which are related to sediment type, particularly particle size and the light reaching the plants. This type of macrophyte survey and classification would elucidate exactly which plants *Najas flexilis* grows in close association with and would indicate the zone within the lake where it would grow.

In contrast to the above classifications, the classification of standing waters in Great Britain (Palmer, 1989) looks at all the macrophytes in the whole lake, and classifies a lake type rather than a community type. Due to this larger survey scale, a greater number of species, representative of a number of communities, would be recorded. This greater species number would give a better estimate of the environment of the lake, because there is a greater chance of including a species that is a good indicator of the environment. However, minor changes in sediment characteristics will not be detected as most lakes contain a range of sediment types. Both the whole lake and the micro-habitat scale approach are used in this study to determine which of these methods of classification would be best for indicating the differences in lake environment and the condition of *Najas flexilis*.

3.1.4 British plant community descriptions and *Najas flexilis*

Despite surveying sites where *Najas flexilis* is known to grow, only Rodwell and Pearsall actually included *Najas flexilis* in their community classifications. *Najas flexilis* occurs in the NVC in community A13, the *Potamogeton perfoliatus-Myriophyllum alterniflorum* community (Rodwell, 1995). *Najas flexilis* is thought to be found equally frequently in both the *Potamogeton berchtoldii* and *Potamogeton filiformis* sub-community. The constant species in this community are *Littorella uniflora* and *Potamogeton gramineus* as well as those named in the association title. Spence (1964) did not include *Najas flexilis* in his associations for macrophyte flora. However, his *Potamogeton gramineus* society is synonymous with A13. A13 is also thought to be represented in standing water type (SWT)

2, 3, and 5 and occasionally 4, in the botanical classification of standing waters in Great Britain (Palmer 1989). *Najas flexilis* however is not mentioned in the SWT descriptions.

Pearsall (1920) places *Najas flexilis* in a consocies of its own, he reports it to be occasionally found with *Potamogeton pusillus, Hydrilla verticillata* and *Nitella flexilis.* Since he only found *Najas flexilis* growing in Esthwaite Water, this community description is based solely on one site. Therefore, it is only an example of a community in which *Najas flexilis* can grow. He also notes that it grows on very fine semi-liquid muds, of low organic content and rich in potassium, which he found nowhere else in the English Lake District. The exclusion of *Najas flexilis* from many of these community descriptions or the inclusion of too few sites where *Najas flexilis* is found, means that hitherto there has been insufficient knowledge to know whether these plant community classifications can be used as a site condition monitoring tool for *Najas flexilis.*

The NVC and SWT will be the British classifications which will be assessed for their suitability as a SCM tool to determine *Najas flexilis* condition. This is because they represent the two different scale approaches to plant community description in lakes. They were also produced after a more widespread geographical survey encompassing sites from England, Scotland and Wales. A wide geographic basis is important as although *Najas flexilis* is thought to be extinct in England at present, SCM will take place in England to determine whether the site improves to a state where reintroduction is possible.

In order for community classifications to be a suitable SCM tool for *Najas flexilis*, the community classification must reflect the underlying environmental conditions that affect the ability of *Najas flexilis* to grow. Ideally *Najas flexilis* would grow in a number of communities, one or more where it could thrive, one or more where it was at risk, and the community would be different again if *Najas flexilis* could not grow at all. Change in the community classification could then be used as an early warning that *Najas flexilis* is at risk before it is lost from a site.

3.2 Methods

3.2.1 Sites

During the course of the study, a total of 52 lakes were investigated, 42 in Scotland (July-September, 1998-2000) and 10 in Ireland (August 2000). All sites with post 1980 records

Table 3.1 The lochs surveyed in this study, the date of survey and the method used. bo denotes boat surveys, sn, snorkel surveys and sh, shoreline survey (grid references for the sites can be found in chapter 1).

Site	Survey method	1988	1999	2000
Mainland Scotland				
Loch a Bhada dharaich	sn & sh	--	--	11/09/00
Loch of Butterstone	bo & sn & sh	03/07/98	16/08/99	--
Loch Clunie	bo & sn & sh	14/07/98	18/08/99	05/08/00
Loch of Craiglush	sn & sh	--	17/08/99	05/08/00
Fingask Loch	sn & sh	--	08/07/99	--
Loch Kindar	bo & sh	24/5/98 18/6/98		
Lindores Loch	sh	--	--	05/08/00
Loch of Lowes	sn & sh	--	16/08/99	05/08/00
Loch Marlee	bo & sn & sh	13/07/98	17/08/99	--
Lake of Menteith	bo & sn & sh	30/07/98 8/10/98	14/10/99	08/08/00
Monk Myre	sn & sh	--	07/07/99	--
Loch Monzievaird	sh	--	--	04/08/00
White Loch	bo & sn & sh	--	08/07/99	--
Inner Isles and Kintyre				
Loch Ballyhaugh	sn & sh	--	24/07/99	--
Loch Fada	sn & sh	--	21/07/99	--
Glenastle Loch	sn & sh	--	19/07/99	25/08/00
Loch Gorm	bo & sh	--	20/07/99	24/08/00
Loch Lossitt	sn & sh	--	20/07/99	25/08/00
Loch nan Gad	sn & sh	07/08/98	--	30/08/00
Loch Poit na h-I	sn & sh	--	22/07/99	--
Tangy Loch	bo & sn & sh	06/08/98	--	29/08/00
Loch an t-Sagairt	sn & sh	--	25/07/99	--
Outer Isles				
Loch Altabrug	sn & sh	--	02/09/99	14/09/00
Loch Bun an Ligidh	sn & sh	17/08/98	29/08/99	13/09/00
Loch nan Capull	sn & sh	21/08/98	01/09/99	13/09/00
Loch nan Cnamh	sn & sh	--	31/08/99	--
Loch Cuilc	sn & sh	17/08/98	--	--
Loch na Cuithe Moire	sn & sh	21/08/98	01/09/99	13/09/00
Loch Druidibeg	bo & sn & sh	18/08/98	31/08/99	13/09/00
North Loch Druidibeg	sn & sh	16/8/98 1/10/98	31/08/98	14/09/00
Loch Eaval	sn & sh	--	30/08/99	--
Loch an Eilean (Drimsdale)	sn & sh	--	02/09/99	--
Loch Eilean a' Ghille-Ruaidh	sn & sh	--	02/09/99	--
Loch an Eilean (S. Boisdale)	sn & sh	--	01/09/99	--
Loch nam Faoileann	bo & sn & sh	19/8/98 1/10/98	01/09/00	--
Loch Gearraidh Mhic Iain	sn & sh	--	02/09/99	--
Loch Grogary	sn & sh	20/08/98	30/08/99	--
Loch a'Mhadaidh	sn & sh	--	31/08/99	--
Mid Loch Ollay	sn & sh	--	29/08/99	14/09/00
Loch Scarie	sn & sh	20/08/98	30/08/99	14/09/00
School House Loch	sn & sh	--	03/09/99	--
Loch with no name (?)	sn & sh	--	02/09/99	14/09/00
Ireland				
Carragh Lough	sn & sh	--	--	17/08/00
Cregduff Lough	sn & sh	--	--	17/08/00
Fin Lough	sh	--	--	16/08/00
Kindrum Lough	sn & sh	--	--	15/08/00
Lough Leane	bo & sh	--	--	18/08/00
Mullaghderg Lough	sn & sh	--	--	15/08/00
Nafeakle Lough	sn & sh	--	--	16/08/00
Pollacappul Lough	sh	--	--	16/08/00
Renvyle Lough	sn & sh	--	--	16/08/00
Shennagh Lough	sn & sh			14/08/00
Shennagh Lough	sn & sh	--	--	14/08/00

for *Najas flexilis* in Scotland were visited (36 sites). In addition 6 sites where no surveys had been carried out post 1980, but where *Najas flexilis* had previously been reported were visited; when surveyed for this study 5 of these appeared now not to contain *Najas flexilis*. All ten Irish loughs visited had previous records for *Najas flexilis* (Table 3.1).

3.2.2 Survey methods

Three basic survey methods were used; boat, shoreline and snorkel surveys. Snorkel surveys were considered the most effective for finding *Najas flexilis*, agreeing with comments in the literature (Capers, 2000), and were adopted as the standard approach wherever feasible. Boats were used when the lakes were particularly large and there was no knowledge of exactly where the plant could be found, this enabled a larger area to be surveyed. When boats were not available, a shoreline search was carried out. A shoreline search included searching the drift and wading into the water as far as safety would allow and throwing grapnels into the deeper water. This method was used by the Nature Conservancy Council when surveying macrophytes for the classification of SWT (Palmer, 1989). The location of *Najas flexilis* communities was determined within a loch from past records where available, see section 2.2.1. It was important to locate *Najas flexilis* within the loch, as the purpose was to describe the community in which it grew.

3.2.2.1 Lake community

In order to ascertain the lake communities in which *Najas flexilis* grew all plants encountered within a lake were recorded on a presence/absence basis. This included submerged and floating leaved plants from shoreline to deep water. Emergent plants were excluded from the analysis for two reasons. Firstly, because of their emergent nature they are often subject to influences not experienced by the open-water vegetation and can therefore skew the results. Secondly, they were not used in the classification of SWT in Britain, to which the communities in this study are compared. Although a representative sample of each loch was surveyed, the entire loch was not surveyed in a detailed manner. Therefore, plants that were scarce within the loch or with a very patchy distribution, not occurring in the vicinity of the *Najas flexilis* community surveyed, may have been missed by the survey. This should not hinder the classification of these communities on a loch basis using the SWT scheme as the classification relies on common and dominant species within a loch.

3.2.2.2 Micro-habitat

The micro-habitat includes only the plants growing in close proximity to *Najas flexilis*. A single micro-habitat was identified and surveyed in each lake, this was at the same site within a lake as the environmental measures were taken. Due to the intrinsic difficulties of surveying deep water plants in low-visibility water, it was decided to collect quantitative samples at the micro-habitat scale by the use of an Ekman grab. This takes 20 x 20 cm^2 samples of the vegetation; three such samples were taken within the micro-habitat. This sampling method does survey a much smaller area than the NVC, however only three samples were taken due to resource, effort and time limitations. In sites where the community was visible it was noted that most species present in the micro-habitat were included in the three Ekman grab samples. Three 20 x 20 cm^2 samples would not be sufficient to represent the community in all ecosystems, however macrophyte communities have been noted by Rodwell (1995) often to be mono-specific even at the larger scale of 2 x 2 m^2. The three Ekman grab samples were sorted into species and dried. The mean dry weight of each species for the three samples was used as the quantitative measure of each species, for that site, at the micro-habitat level. This data is not appropriate for classification using the SWT method as even species that are common within a lake may be excluded at such a small scale. Neither is it the purpose of this data to classify lake type, but specific community type, therefore the NVC should be a more appropriate classification system to use. These samples were only collected from the Scottish lochs where *Najas flexilis* was found. If *Najas flexilis* was not found, the micro-habitat where it grew could not be assumed, thus collection of such samples would have been of no value for this exercise.

3.2.3 Environmental measures

In order to determine the underlying environment of the different communities a number of environmental measures were taken at each site. These measures are the same as those in section 2.2.3. Exposure was calculated using the same method as in section 2.2.3. This was only calculated for the micro-habitat sites as exposure can only be calculated for a fixed point not a whole lake.

3.2.4 Data analysis

TWINSPAN (Hill, 1979) was used to group the sites according to their species composition. Detrended Correspondence Analysis (DCA) (Hill & Gauch, 1980) was used to look at the variation in species composition of the sites in multidimensional space. The resulting groups were classified according to SWT (Palmer, 1989) and the NVC (Rodwell,

1995) with the use of the MATCH program (Malloch, 1996). The quantitative environmental measures do not have homogeneous variance, this is because the lower limit of detectability is surpassed in some of these measures. Consequently, nonparametric tests were used to compare the medians of the environmental variables between groups. The Kruskal-Wallis test was used to compare the environmental measures of each of the lake scale TWINSPAN groups. The nonparametric multiple comparison test of Dunn (1964) was then used on this data (Zar, 1999). As the TWINSPAN groups created from the micro-habitat scale survey contain sample sizes of only n = 2, Mann-Whitney U tests were used to independently compare the environmental conditions in these groups.

3.3 Results

3.3.1 Lake communities

3.3.1.1 Community groupings

Both TWINSPAN and DCA show that *Najas flexilis* can be found in a number of different whole lake communities. This is potentially useful for a SCM tool, if these communities correspond to conditions suitable for *Najas flexilis* growth. TWINSPAN produced 4 main site-groups (Table 3.2a). Only four groups were produced as all sites that support *Najas flexilis* are intrinsically similar. The cut off criteria for these groups were that the groups were still distinguishable from each other in terms of their floristic composition and that they made ecological sense, the same applies for the TWINSPAN groups produced from the micro-habitat data. All of these groups contain sites from more than one of the geographical areas of mainland, Inner Isles, Outer Isles and Ireland. This suggests that the difference in floristic composition between the groups is not due to geographical reasons. The species occurring in the TWINSPAN groups are shown in the floristic table (Table 3.3). These TWINSPAN groups have been classified according to SWT, the classification of these groups are shown in Table 3.4.

The DCA for the whole lake species assemblages (Fig 3.2) shows that although different communities can be identified their comparative closeness within the diagram indicates they are not greatly differentiated. The DCA axis 1, is 7 standard deviation units (SD) long and axis 2 is 7.5 SD long, this suggests a complete turnover of species along these axis. However the sites are clustered within 3 SD of each other, this is because the sites as well as having different species within them also have a number of species in common, particularly *Najas flexilis,* as well as species which are common in a number of plant

associations. The sites that appear most separated from the rest are in TWINSPAN group 4, five out of the seven sites within this group are sites where *Najas flexilis* is thought to be extinct. The cumulative percentage variance of the species explained by the first two axes is only 16 %.

3.3.1.2 Species composition

The main split in the groupings created by TWINSPAN on the lake scale data is between groups 1 and 2, and groups 3 and 4. Groups 1 and 2 most frequently support species associated with a more oligotrophic environment, primarily the isoetids; *Littorella uniflora, Isoetes lacustris* and *Lobelia dortmanna* as well as other species such as *Juncus bulbosus* and *Sparganium angustifolium*. Groups 3 and 4 contain less of these species and more frequently contain species most commonly found in more eutrophic environments, such as *Callitriche hermaphroditica* and *Elodea* spp. (if the area has been invaded by the latter). These are also the sites where the *Potamogeton* spp. associated with higher nutrient concentrations can be found, such as *Potamogeton pectinatus*, *Potamogeton lucens* and *Potamogeton obtusifolius* (Palmer, 1989). Within these groups differences are also apparent, group 2 is more dominated by *Potamogeton perfoliatus* and *Potamogeton gramineus* than group 1, suggesting a slightly more eutrophic group 2. Group 4 contains *Persicaria amphibia*, which is not found in group 3, and less *Najas flexilis*, suggesting group 4 is the most eutrophic. This is represented by the SWTs assigned to each group (Table 3.4).

3.3.1.3 Environmental conditions

The differences in water conditions between these community groupings are shown in Table 3.5a. Group 1 is constantly the most nutrient poor and group 4 is the most nutrient rich, this is shown by significantly different levels of total oxidised nitrogen and dissolved reactive phosphate. Other factors that are significantly different between the two groups are pH and alkalinity, both of which also tend to show higher values at higher trophic states. Groups 2 and 3 have a more intermediate nutrient status, this is as the species composition suggested. The underlying sediment chemistry is more confusing (Table 3.5b), group 4 is still consistently the most rich in phosphate followed by group 3, group 1 seems then to be more phosphate rich than group 2. However, the latter two groups are not significantly different ($p>0.05$). Calcium levels are also highest in group 4, but group 3 has the lowest levels with groups 1 and 2 as intermediates. Iron is highest in group 1 and lowest in groups 2 and 3

with intermediate levels in group 4. These differences in the environmental conditions are potentially very useful for predicting *Najas flexilis* growth as it is the nutrient levels and calcium concentration which appear to be important in predicting its success.

3.3.2. Micro-habitat

3.3.2.1 Community groupings

Both TWINSPAN and DCA show that more than one community at the micro-habitat scale contains *Najas flexilis,* again suggesting that this approach could be a useful SCM tool. Three main TWINSPAN site-groups were produced with this approach (Table 3.2b). Each group contains sites from more than one geographical area, suggesting geographical reasons do not determine floristic composition within a group. The species occurring in the TWINSPAN groups are shown in the floristic table (Table 3.3). These TWINSPAN groups have been classified according to the NVC (Table 3.4). The coefficients provided by the MATCH programme for the goodness of fit for these communities to NVC types are also shown in Table 3.4. A match coefficient of 100 is a perfect match and 0 shows no similarity.

The DCA axis 1 and 2, on the micro-habitat species assemblage data (Fig. 3.2) explain more of the species variance than the same analysis on the whole lake species assemblages, 23.6% of the micro-habitat species variance is explained in comparison to 16% of the lake community species variance . These sites are slightly more separated on both the axis than the lake scale data, most probably because the common species, or those with a wider ecological niche which would have been present in most of the lakes, would not necessarily be found in all the micro-habitats making the micro-habitat a more definable unit. Despite the fact that the sites are spread over 4.5 SD on axis 1, showing that sites at either end of the axis are quite dissimilar, the sites in group 2 & 3 appear to be a continuous range rather than 2 distinct groups.

Table 3.2 Sites contained in each of the TWINSPAN groups for the lake scale (a) and micro-habitat scale (b). Where *E* = Ireland, *I* = Inner Isles and Kintyre, *M* = Mainland Scotland and *O* = Outer Isles.

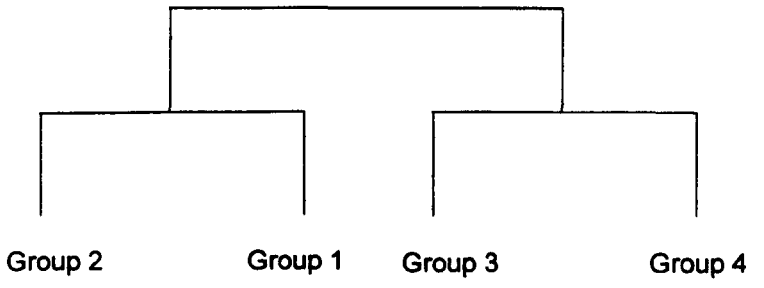
(a)

Group 1	**Group 2**	**Group 3**	**Group 4**
Loch nan Capull *O*	Loch na Cuithe Moire *O*	Loch Grogary *O*	Loch Clunie *M*
Loch an t-Sagairt *I*	Glenastle Loch *I*	Loch Scarie *O*	Lough Leane *E*
Nafeakle Loch *E*	Mid Loch Ollay *O*	Tangy Loch *I*	Loch Marlee *M*
Loch a Bhada dharaich *M*	Loch Eaval *O*	Loch Lossitt *I*	Monk Myre *M*
Loch Altabrug *O*	Loch Gearraidh Mhic Iain *O*	Loch nan Cnamh *O*	White Loch *M*
Loch Fada *I*	Loch of Craiglush *M*	Loch an Eileen (S. Boisdale) *O*	Lindores Loch *M*
Renvyle Lough *E*	Loch an Eilean (Drimsdale) *O*	Fingask Loch *M*	Loch Monzievaird *M*
Fin Lough *E*	Loch Poit na h-I *I*	Loch of Lowes *M*	
Carragh Lough *E*	Loch Bun an Ligidh *O*	Loch of Butterstone *M*	
Shennagh Lough *E*	Loch Druidibeg *O*	Lake of Menteith *M*	
Kindrum Lough *E*	Loch nan Gad *I*		
Cregduff Lough *E*	School House Loch *O*		
Loch nam Faoileann *O*	Loch Gorm *I*		
Loch Kindar *M*	North loch Druidibeg *O*		
Mullaghderg Lough *E*	Loch with no name (?) *O*		
Pollacappul *E*	Loch Eileen a' Ghille-Ruaidh *O*		
	Loch Ballyhaugh *I*		
	Loch a'Mhadaidh *O*		

(b)

Group 1	**Group2**	**Group 3**
Loch Eaval *O*	Loch of Craiglush *M*	Loch Grogary *O*
Loch Poit na h-I *I*	Loch Fada *I*	Loch a'Mhadaidh *O*
	Loch an t-Sagairt *I*	Loch Gorm *I*
	Loch Bun an Ligidh *O*	Loch nan Capull *O*
	Loch na Cuithe Moire *O*	Loch Scarie *O*
	Loch an Eilean (Drimsdale) *O*	Loch Ballyhaugh *I*
	Glenastle Loch *I*	Loch Druidibeg *O*
	Loch Gearraidh Mhic Iain *O*	School House Loch *O*
	Loch Eileen a' Ghille-Ruaidh *O*	Loch nan Gad *I*
	Mid Loch Ollay *O*	Loch Altabrug *O*
	North loch Druidibeg *O*	Loch nam Faoileann *O*
		Loch Lossitt *I*
		Loch of Butterstone *M*
		Loch nan Cnamh *O*
		Loch with no name (?) *O*
		Loch of Lowes *M*
		Lake of Menteith *M*
		Loch Clunie *M*

a)



b)

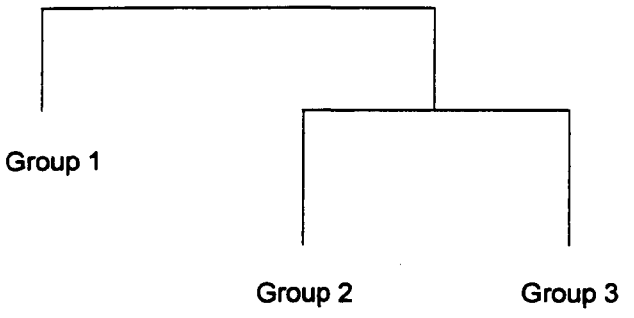
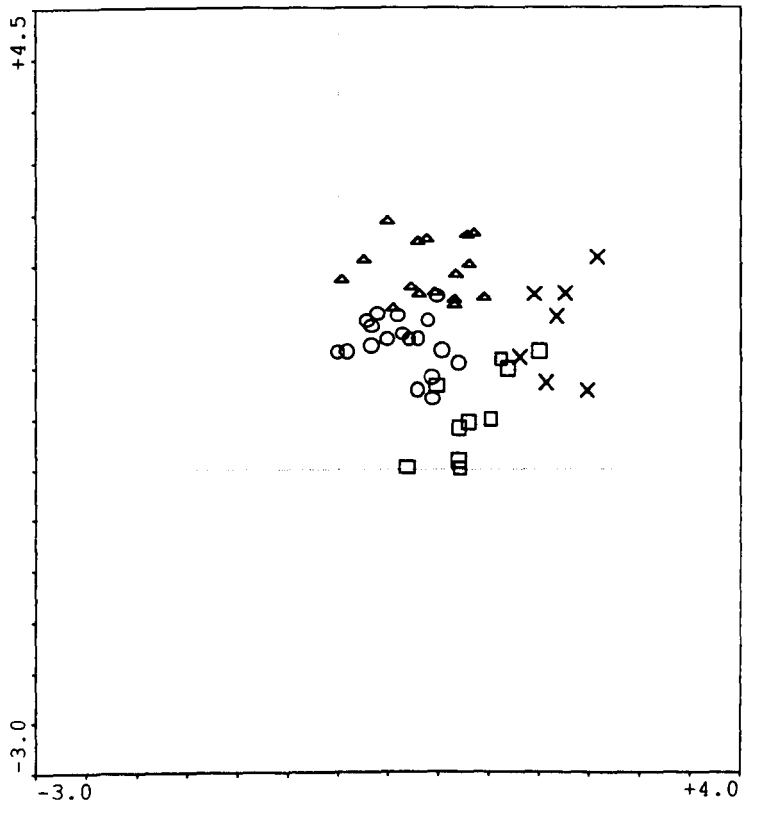


Fig. 3.1 Dendrogram showing the separation of TWINSPAN groups for a) the lake scale communities and b) the micro-habitat communities.



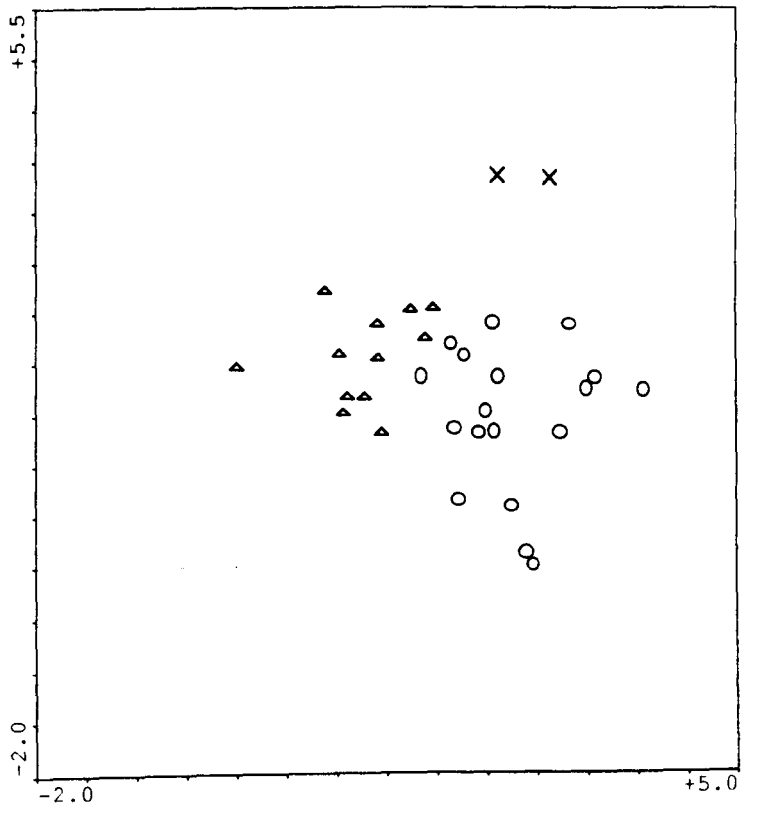


Fig 3.2 DCA showing site groupings according to TWINSPAN on a) the lake scale species composition where; △ represents group 1, O group 2, □ group 3 and × group 4 and b) the micro-habitat scale species composition where; × represents group 1, △ group 2 and O group 3.

3.3.2.2 Species composition

The splits between the groups created on the micro-habitat species assemblages are more clear-cut than those for the whole lake species assemblages. The groups obtained from the micro-habitat surveys did not all contain *Najas flexilis.* When *Najas flexilis* was dominant within the community it was always obtained within the samples, however due to the small size of the vegetation samples taken, this was not the case when it was an occasional within the community. This is because it was the community that was sampled and not *Najas flexilis.* This illustrates that only the dominant species within the community would necessarily be found in the samples, and therefore these samples are classified based on the dominant species. Both group 2 and 3 did constantly contain *Najas flexilis* (Table 3.3b). Group 2 most frequently contains the isoetids, *Isoetes lacustris*, *Lobelia dortmanna* and *Littorella uniflora* as well as *Juncus bulbosus*, whilst group 3 most frequently contains

Table 3.3 Floristic table showing the frequency of plant occurrence within the groups created on (a) the lake scale and (b) the micro-habitat scale. V = 80-100%, IV = 60-80%, III = 40-60%, II = 20-40%, I = <10%.

(a)

	Group 1 n = 16	**Group 2 n = 18**	**Group 3 n = 10**	**Group 4 n = 7**
Juncus bulbosus	V	V	II	
Littorella uniflora	V	V	V	V
Najas flexilis	V	V	V	II
Potamogeton natans	V	IV		
Isoetes lacustris	IV	IV	II	II
Potamogeton berchtoldii	IV	I	V	III
Elatine hexandra	III	I	I	I
Fontinalis antipyretica	III	I	II	
Lobelia dortmanna	III	IV		
Menyanthes trifoliata	III	II		II
Myriophyllum alterniflorum	III	IV	V	IV
Nitella spp.	III	I	I	I
Nymphaea alba	III	I	I	I
Potamogeton perfoliatus	III	V	V	III
Sparganium angustifolium	III	III	I	II
Chara spp.	II	IV	III	V
Eleocharis palustris	II	I		II
Elodea canadensis	II	I	II	V
Potamogeton x nitens	II	I	II	I
Ranunculus aquatilis	II			III
Ranunculus flammula	II			I
Utricularia vulgaris	II	II	I	
Eriocaulon aquaticum	II			
Baldellia ranunculoides	I	I		
Callitriche hamulata	I	I	I	
Callitriche hermaphroditica	I	II	IV	V
Callitriche stagnalis	I			
Eleocharis acicularis	I	I	II	II
Eleogiton fluitans	I			
Elodea nuttallii	I		III	
Isoetes setacea	I			

Lemna minor	I			II
Nuphar lutea	I		I	III
Potamogeton alpinus	I			II
Potamogeton crispus	I		I	II
Potamogeton gramineus	I	IV	III	III
Potamogeton polygonifolius	I	I		
Potamogeton praelongus	I	I	I	
Potamogeton pusillus	I		I	II
Potamogeton rutilus	I	I	II	
Subularia aquatica	I			I
Persicaria amphibia	I			II
Hydrilla verticillata	I			
Apium inundatum		I		
Myriophyllum spicatum		I	I	
Potamogeton lucens		I		II
Potamogeton obtusifolius				III
Potamogeton pectinatus		I	III	
Potamogeton x zizii		I		II
Sparganium erectum		I		I
Zannichellia palustris			I	
Ceratophyllum demersum				I

(b)

	Group 1 n = 2	**Group 2 n = 11**	**Group 3 n = 18**
Isoetes lacustris		IV	I
Juncus bulbosus	III	IV	II
Najas flexilis		IV	IV
Littorella uniflora		III	I
Potamogeton gramineus	V	III	II
Lobelia dortmanna		II	
Myriophyllum alterniflorum		II	I
Callitriche hermaphroditica		I	II
Chara spp.		I	III
Elodea canadensis		I	II
Potamogeton berchtoldii		I	IV
Potamogeton perfoliatus		I	IV
Sparganium angustifolium	III	I	II
Elatine hexandra			I
Eleocharis acicularis			I
Elodea nuttallii			I
Fontinalis antipyretica	V		I
Nitella spp.	III		I
Potamogeton lucens	III		
Potamogeton pectinatus			I
Potamogeton rutilus	III		I
Utricularia vulgaris	III		
Potamogeton pusillus			I

Chara spp., *Potamogeton berchtoldii* and *Potamogeton perfoliatus*. Group 1 is grouped on the lack of these otherwise constant species, the high frequency measurements for this group are artefacts of the small sample size, only two. These groupings are all classified as separate NVC types (Table 3.4b).

3.3.2.3 Environmental conditions

The only environmental variable that is significantly different ($p<0.05$) between groups is the light extinction coefficient where group 3 communities inhabit clearer water than group 2 (Table 3.6).

Table 3.4 (a) Standing water type classification of the groups created from the lake scale species composition data, (b) NVC of the groups created from the micro-habitat species composition data given with MATCH coefficient.

(a)

	Group 1	Group 2	Group 3	Group 4
Palmer	SWT 3	SWT 4	SWT 5a	SWT 5a

(b)

	Group 1	Group 2	Group 3
NVC	A24a	A23	A13
Match coefficient	45.4%	64.6 %	66.3%

Table 3.5 Environmental conditions of the lake scale plant community groups (a) water measurements, (b) sediment measurements. Values with different letters are significantly different ($p<0.05$; Kruskal-Wallis test and Dunn multiple comparisons test).

(a)

	Group 1	Group 2	Group 3	Group 4
pH	6.46 - 7.97^{a} (n = 16)	6.84 - 8.26^{ab} (n = 18)	7.04 - 8.78^{b} (n = 10)	7.11 - 9.01^{b} (n = 7)
Median	7.15	7.44	7.77	7.95
Conductivity (us/cm)	54.9 - 447 (n = 16)	97 - 405 (n = 18)	77 - 370 (n = 10)	143 - 410 (n = 7)
Median	179.5	270.5	278	214
Light extinction coefficient	0.78 - 7.32 (n = 16)	1.11 - 6.23(n = 18)	0.53 - 5.18 (n = 10)	0.91 - 4.32 (n = 7)
Median	2.608668	2.000661	0.940007	1.393594
Alkalinity (mg/l)	4.91 - 65.7^{a} (n = 12)	8.61 - 69.3^{ab}(n = 12)	9.7 - 69.71^{ab} (n = 7)	30.16 - 98.66^{b} (n = 3)
Median	16.28	22.01	32.4	72.65
Ca (mg/l)	1.32 - 22.4 (n = 13)	3.88 - 33.4 (n = 12)	8.26 - 25.8 (n = 7)	11.42 - 24.72 (n = 3)
Median	7.96	8.78	13.14	21.7
Fe (mg/l)	<0.03 - 0.99 (n = 13)	<0.003 - 1.69 (n = 12)	<0.03 - 1.51 (n = 7)	0.04 - 0.35 (n = 3)
Median	0.22	0.21	0.135	0.08
Mg (mg/l)	1.34 - 7.2 (n = 13)	2.8 - 6.2 (n = 12)	1.34 - 8.8 (n = 7)	2.08 - 12 (n = 3)
Median	4.4	4.8	6.4	8.8
N-NH_3 (mg/l)	<0.03 - 0.1 (n = 13)	<0.03 - 0.08 (n = 12)	<0.03 - 0.1 (n = 9)	<0.03 - 0.28 (n = 5)
Median	0.04	<0.03	<0.03	0.056
N-TON (mg/l)	<0.01 - 0.03^{a} (n = 13)	<0.01^{a} (n = 12)	<0.01 - 0.11^{a} (n = 9)	<0.01 - 2.64^{b} (n = 5)
Median	<0.01	<0.01	<0.01	0.88
P-DRP (PO_4) (mg/l)	<0.001^{a} (n = 13)	<0.001 - 0.005^{ab} (n = 12)	<0.001 - 0.004^{ab} (n = 9)	<0.001 - 0.074^{b} (n = 5)
Median	<0.001	<0.001	<0.001	0.023
P-TP (mg/l)	<0.001 - 0.021 (n = 13)	<0.001 - 0.02 (n = 12)	<0.001 - 0.026 (n = 7)	0.014 - 0.16 (n = 3)
Median	0.01	0.0085	0.013	0.056
K (mg/l)	0.3 - 2.82 (n = 13)	0.16 - 2.14 (n = 12)	0.3 - 1.9 (n = 9)	1.2 - 2 (n = 5)
Median	0.94	0.9	1.1	1.52

(b)

	Group 1	Group 2	Group 3	Group 4
Available P (mg/l)	0.3 - 17.3[ab] (n = 15)	0.3 - 3.6[a] (n = 17)	1.1 - 84.4[bc] (n = 10)	3.6 - 72.9[c] (n = 7)
Median	1.3	1.3	2.8	16.65
Available K (mg/l)	4 - 166 (n = 5)	4 - 136 (n = 17)	9.5 - 104 (n = 9)	28.3 - 95.5 (n = 4)
Median	15.7	37.5	29	77.1
Extractable Mg (mg/l)	50.9 - 344 (n = 5)	17.3 - 388 (n = 18)	16.3 - 302 (n = 9)	54.1 - 189 (n = 4)
Median	211	141	66.4	106.5
Extractable Ca (mg/l)	145 - 5720[ab] (n = 15)	198 - 1930[ab] (n = 18)	1.8 - 1550[a] (n = 10)	805 - 331000[b] (n = 7)
Median	816	896	544	3180
Total Fe (mg/kg)	516 - 168000[b] (n = 15)	94.4 - 11900[a] (n = 18)	105 - 14500[a] (n = 10)	123 - 11300[ab] (n = 7)
Median	890	786	570	187
Total N (mg/kg)	290 - 142000 (n = 15)	240 - 89200 (n = 18)	350 - 27100 (n = 10)	600 - 40800 (n = 7)
Median	15100	6220	2120	6255
Extractable Na (mg/l)	34.2 - 205 (n = 4)	17.4 - 300 (n = 16)	8.3 - 239 (n = 9)	20.5 - 72.5 (n = 4)
Median	63.25	76.85	35.6	33.8

Table 3.6 Environmental conditions of the micro-habitat scale plant community groups (a) water measurements, (b) sediment measurements. Values with * are significantly different ($p<0.05$; Mann-Whitney U test).

(a)

	Group 1	Group 2	Group 3
pH	7.2 - 8.2 (n = 2)	6.84 - 8.25 (n = 11)	7.04 - 8.3 (n = 18)
Median	7.7	7.3	7.54
Conductivity (us/cm)	269 - 345 (n = 2)	97 - 398 (n = 11)	77 - 447 (n = 18)
Median	307	235	232
Light extinction coefficient	1.11 - 3.21 (n = 2)	1.23 - 6.23 (n = 11)*	0.53 - 7.32 (n = 18)*
Median	2.169054	2.978303	1.767662
Alkalinity (mg/l)	69.3 (n = 1)	8.61 - 40.95 (n = 7)	9.7 - 69.71 (n = 12)
Median	69.3	19.15	25.06
Ca (mg/l)	33.4 (n = 1)	3.88 - 15.86 (n = 7)	5.72 - 25.8 (n = 12)
Median	33.4	7.98	9.82
Fe (mg/l)	0.13 (n = 1)	0.003 - 0.94 (n = 7)	0.003 - 1.69 (n = 12)
Median	0.13	0.21	0.15
Mg (mg/l)	6.2 (n = 1)	2.8 - 5.6 (n = 7)	1.34 - 8.8 (n = 12)
Median	6.2	5.2	4.8
N-NH_3 (mg/l)	<0.03 (n = 1)	<0.03 - 0.08 (n = 7)	<0.03 - 0.1 (n = 15)
Median	<0.03	<0.03	<0.03
N-TON (mg/l)	<0.01 (n = 1)	<0.01 (n = 7)	<0.01 - 1.369 (n = 15)
Median	<0.01	<0.01	<0.01
P-DRP (PO_4) (mg/l)	<0.001 (n = 1)	<0.001 - 0.004 (n = 7)	<0.001 - 0.005 (n = 15)
Median	<0.001	<0.001	<0.001
P-TP (mg/l)	<0.001 (n = 1)	<0.001 - 0.013 (n = 7)	<0.001 - 0.026 (n= 12)
Median	<0.001	0.003	0.0105
K (mg/l)	2.14 (n = 1)	0.52 - 2.02 (n = 7)	0.54 - 2.02 (n = 15)
Median	2.14	0.86	1.2

(b)

	Group 1	Group 2	Group 3
Available P (mg/l)	1.9 -2.2 (n=2)	0.3 - 3.6 (n = 11)	0.5 - 9.4 (n = 18)
Median	2.05	1.3	2.6
Available K (mg/l)	38.2 - 74.5 (n = 2)	4 - 87.1 (n = 11)	8 - 166 (n = 17)
Median	56.35	37.5	28
Extractable Mg (mg/l)	151 - 92.4 (n = 2)	28.7 - 308 (n = 11)	17.3 - 388 (n = 17)
Median	121.7	77.6	87
Extractable Ca (mg/l)	600 - 1610 (n=2)	290 - 1800 (n = 11)	253 - 1930 (n = 18)
Median	1105	816	1019
Total Fe (mg/kg)	190 - 1630 (n = 2)	126 - 7780 (n = 11)	121 - 11900 (n = 18)
Median	910	578	875
Total N (mg/kg)	590 - 11400 (n = 2)	350 - 38100 (n = 11)	350 - 142000 (n = 18)
Median	5995	10200	8960
Extractable Na (mg/l)	41.6 - 145 (n = 2)	21.1 - 257 (n = 11)	13 - 300 (n = 15)
Median	93.3	72	39.7
Exposure	1.58 - 2.13 (n = 2)	0.58 - 2.73 (n = 11)	0.39 - 4.52 (n = 15)
Median	1.85	0.94	1.26

3.4 Discussion

3.4.1 The ability of the lake scale qualitative approach and the micro-habitat scale quantitative approach to illustrate the different lake conditions.

Both approaches produce multiple community groups that could be used as site condition monitoring tools. Both sets of communities are also found to reflect the environment where they grow, which is to be expected. However, the two approaches elucidate very different aspects of the environment. It could be expected that because the micro-habitat approach looks at a finer scale and includes information on the quantity of a species instead of its presence or absence it should produce a finer scale result, for example, a greater number of communities representing a number of different environments. Instead, in this case, this scale approach only elucidated a difference in light. As predicted, this approach appears to elucidate the different zonations within a lake, but surprisingly it failed to elucidate differences in any other environmental conditions.

The very small scale 20 x 20 cm^2 plant collection for the micro-habitat community may have limited the effectiveness of this approach. Previous attempts at macrophyte classification schemes have used 2x2m^2 or 4x4m^2 quadrats. Instead, in this study we replicated the smaller samples three times to increase the chance of collecting more species. However, macrophytes often grow in monospecific stands or species poor communities much greater in dimension than 4x4m^2 (Rodwell, 1995). Therefore, although increasing the quadrat size would increase the chances of collecting more species a very large quadrat would be required to collect many more species. It is of interest that the NVC, which used a quantitative quadrat-based approach produced classifications of communities which existed in a number of the SWT types, which uses a semi-quantitative lake scale approach.

In contrast, the whole lake approach elucidated the differences in several environmental conditions including pH and phosphorus, which represent acidification and eutrophication, the greatest threats to vegetation in north European softwater lakes (Murphy, 2002). These are the kind of environmental changes that threaten *Najas flexilis*, which site condition monitoring needs to detect (see sections 2.3 & 2.4). This greater ability to detect the environmental differences is due to the greater number of species encountered by surveying a greater area, thus increasing the chance of finding a species that is a good environmental indicator. The level of detail lost by using a qualitative rather than a quantitative technique does not appear to have hindered this technique. This is important, as accurate quantitative

measures of deep water plants are difficult to acquire without snorkelling or SCUBA diving. This is because the quantity of a macrophyte captured on a rake or grapnel is only partly dependent on its abundance. It is also greatly effected by its structure; whether it is likely to snared on the device and how firmly it is rooted in the sediment (Capers, 2000, James, personal communication & personal observation).

3.4.2 Standing Water Type and National Vegetation Classification. How well do these classifications fit the data collected?

Standing Water Type appears to fit the data collected in this study well, whilst the NVC fitted groups 2 and 3 reasonably well and group 1 not so well.

3.4.2.1 Standing Water Type

Groups 1-4 are generally very similar in species composition to the SWTs into which they have been classified, however there are exceptions. All groups contain *Najas flexilis,* which is not included in the SWT classifications. This is not surprising, as due to the rarity of *Najas flexilis* it would not have frequently been found in general lake surveys, compared to this study where lakes containing the plant were purposefully visited and the plant sought out. The other main differences between the groups and their assigned SWT are that group 1 contains a greater quantity of *Potamogeton berchtoldii* than SWT 3. The difference between group 2 and SWT 4 is a greater quantity of *Isoetes lacustris* and *Lobelia dortmanna* in group 2. Group 3 is a good match for SWT 5 with no great differences between them. Group 4 is different to SWT 5 in that it contains a greater amount of *Chara* spp.

The SWT study (Palmer, 1989) provides some indication of the differences in the chemistry of the lakes studied. This means that the environment found to be representative of the groups in this study can be compared to the environment found to be representative of the SWT, which the group has been classified as. Standing Water Type 3 is the most oligotrophic SWT in this study. Sites classified as SWT 3 would most likely have a pH between 6 and 7 and alkalinities of 2-30mg/l: lakes in this group are often on igneous and metamorphic rocks. When compared to the environmental measures in group 1 (Table 3.5) it can be seen that although the ranges include higher measurements than these, the medians are reasonably similar. The median alkalinity measurement is in the middle of the suggested alkalinity range for this type of vegetation, however pH is slightly higher, this maybe because these measurements are based on a single pH reading in summertime.

Palmer (1989) states that SWT 4 is a wide ranging category which represents lochs with a variety of influences, for example machair lochs where the base poor waters run off the peatlands into a more base rich loch overlying shell sand or other calcareous material. This variety of influences on loch chemistry can lead to plants from both base rich and base poor associations coexisting within a loch. Many of the lochs categorised as SWT 4 in this study are machair lochs from the Western Isles of Scotland. The environmental measures from the sites assigned to this SWT, group 2, do not seem to show a wider breadth of environmental conditions, but do show a slight increase in both pH and alkalinity from group 1 assigned to SWT 3.

Both groups 3 and 4 were classified as SWT 5. Standing Water Type 5 is considered typical of mesotrophic conditions. These SWTs typically have a higher pH and alkalinity than SWT3 and sometimes SWT4 (only sometimes due to the wide ranging underlying environment in SWT 4). Both Group 3 and 4 fit with this trend of increasing pH and alkalinity. The environmental conditions represented in group 3 seem to be those most frequently found to support SWT 5. Whereas the environmental conditions found in group 4 sites appear to be at the extreme end of those typically found to support SWT 5. This can be seen by comparing the alkalinity measures. The SWT 5 sites most frequently have an alkalinity that ranges between 10-30mg/l. The median alkalinity for group 3 is 32.5mg/l. The highest alkalinity range found to support SWT 5 vegetation is 30-100mg/l. The median alkalinity for group 4 is 72.65 mg/l.

Standing water type appears to describe similar floristic and environmental groups to those found in this study. However SWT 5 has been split into two further groups in this study with group 3 being a typical mesotrophic lake and group 4 being at the most eutrophic extreme for the SWT 5 category.

3.4.2.2 National Vegetation Classification

Three NVC community types were found in association with *Najas flexilis,* A13, *Potamogeton perfoliatus-Myriophyllum alterniflorum,* A23, the *Isoetes lacustris/setacea* community and A24, the *Juncus bulbosus* community, illustrating that the plant can thrive in more than one community type contrary to Pearsall (1920) and Rodwell (1995). These classifications fit the data from this study to varying degrees.

Group 3, A13

Group 3 is most similar to NVC type A13, however it is not a perfect match. The dominants of community type A13 are constantly under recorded in group 3 with the exception of *Potamogeton perfoliatus* and *Potamogeton berchtoldii.* The following all have a much higher constancy score in A13 than group 3; *Myriophyllum alterniflorum, Littorella uniflora,* and *Potamogeton gramineus.* The under representation of these species is probably due to the smaller scale of sampling in this study and the domination of the communities in many cases by *Najas flexilis. Najas flexilis* is over represented in group 3 compared to A13, this can be expected due to the fact that it is a rare plant so would not have been encountered as many times in a general survey, as in a study focused on this species.

A13 is the only community within the NVC to be reported to contain *Najas flexilis.* It is representative of a wide habitat covering shallow to quite deep, mesotrophic and rather base poor waters, which are still or gently flowing with fine to coarse mineral beds, but not peats. The community type is generally confined to the north and west of Britain and occurs in pools, lakes and the middle to lower reaches of rivers (Rodwell, 1995).

Group 2 A23

The Match programme provides a good match between the group 2 data and the NVC type A23. However, the sample data contain a greater abundance and frequency of *Juncus bulbosus* and also contain *Potamogeton gramineus* as a dominant, this species is not mentioned in the NVC classification. This suggests that the community described by the sample data is not strictly the same as the NVC classification. The *Isoetes* community described in Rodwell (1995) is typically found on barren, stony substrates, in clear, deep waters of less fertile lakes through north west Britain. *Najas flexilis* was never found on stony substrate in this study, but always on fine silt. The water was not necessarily clear either, particularly in those lochs where the water was stained by the peat. These lochs clearly represent the oligotrophic end of the range of *Najas flexilis* communities, but with occasional species such as *Callitriche hermaphroditica* and *Elodea* spp. occurring in both the description by Rodwell and the equivalent *Isoetes* society described by Spence (1964) and such species being present in group 2, it suggests that it may not obligatorily be so very oligotrophic.

Group 1 A24

The species found in group 1 are not a good match to NVC type A24. This is probably due to the low sample number making meaningful classification difficult. These two samples are obviously outliers from the other two communities (Fig 3.3), this seems to be due to the lack of constant species found in the other 2 groups, rather than the presence of different species within group 1. They are unlikely to both be members of a *Juncus bulbosus* community when only one site contains this species, the misleadingly high frequencies is due to the small sample size. Instead, these outliers emphasise that although the presence of specific species of macrophytes or their communities can indicate suitable conditions for *Najas flexilis* growth, the absence of one or two of these species does not make the site necessarily unsuitable.

3.4.3 How well would SWT or the NVC predict *Najas flexilis* success?

3.4.3.1 Standing Water Type

Standing Water Type communities vary across the sites where *Najas flexilis* grows and this appears to coincide with trends in trophic state. This indicates that *Najas flexilis* does not appear to be restricted to just one "ideal" plant community, but occurs across a range. Standing Water Type 3 and SWT 5 contained sites where *Najas flexilis* is thought to be extinct. This shows that although *Najas flexilis* can grow in SWT 3-5 it is those at the most oligotrophic and eutrophic extremes of its range that are most at risk. This would appear to agree with Murphy (2002 in press) that the greatest threats to vegetation in north European softwater lakes are eutrophication and acidification.

Both groups 3 and 4 were identified as SWT 5, yet group 4 appeared to be at the eutrophic limit for this SWT and a reasonably unsuitable environment for *Najas flexilis* growth. SWT 3 is obviously at the opposite end of the scale and group 1, assigned to this SWT, contains the 2 extinct oligotrophic sites. However many other sites assigned to this group contain healthy *Najas flexilis* populations. A more sensitive scheme than SWT would be needed to indicate exactly which *Najas flexilis* sites are most at risk. Therefore, it appears that although plant communities can indicate the groups that will contain at risk sites many of the sites within such a group will also contain healthy *Najas flexilis* populations. This is because many plants appear to have a wider niche than *Najas flexilis*, so these species cannot be used as indicators for *Najas flexilis* condition. Therefore, a lake scale approach to

community classification could indicate a group of sites that were likely to contain at risk sites for *Najas flexilis*. However it would be unable to pin point exactly which sites were at risk.

3.4.3.2 NVC

The NVC communities in which *Najas flexilis* is found illustrates which depth zones it occupies within the lake. The *Isoetes* community is often the deepest vegetated zone within the lake (Rodwell, 1995; Spence, 1964). The *Potamogeton perfoliatus-Myriophyllum alterniflorum* community is thought to represent an intermediate zone with most commonly a *Littorella-Lobelia* community between it and the shore. This occurs when the shoreline is exposed and therefore made up of coarse material rather than silt. When the first sediment type encountered after leaving the shore is silt, A13 can be the first community encountered. This is particularly the case in small lochs or sheltered bays. In deeper water, this community can be replaced by *Nitella* spp. as seen in the Lake of Menteith or *Chara* spp. as in Pollacapull Lough. It can also be replaced by *Isoetes* communities, containing abundant *Najas flexilis* as seen in Loch Druidibeg. This explains the significant difference found in the light extinction coefficient between group 2, A23 and group 3, A13. However, *Najas flexilis* appears to grow equally well in both these communities, but not the *Littorella-Lobelia* community, which grows on the gravelly substrate. This indicates that the positioning of *Najas flexilis* within a loch is more often due to sediment type (and probably competition, this will be discussed in Chapter 4) than by light, within the vegetated region of the lake, as the plant occurs in communities in the shallows and the depths wherever there is fine sediment. Fine sediment will only occur where exposure is low (see section 1.4.4). Therefore, it is likely that both A23 and A13 are in areas with lower exposure than the *Littorella-Lobelia* community. Exposure may control *Najas flexilis* growth directly through disturbance or indirectly through its effect on sediment type.

As an indicator of the condition of a lake and its favourability for *Najas flexilis* growth, use of the NVC at the micro-habitat scale does not appear to be the best method. This is because the species that can grow in association with *Najas flexilis* appear to have wider ranges in which they can grow than *Najas flexilis*. As a result, no differences in the environmental conditions that appear to control *Najas flexilis* growth are elucidated by looking at the vegetation at the micro-habitat scale. However, it does elucidate the zones within a lake that can support *Najas flexilis*. NVC type A13 is thought to be represented in

lakes, which are SWT 2, 3, 5, and occasionally 4 and NVC type A23 represented in SWT 2 and 3. This illustrates the wide range of environments incorporated into a single NVC community. This wide environmental range makes the NVC a poor tool for indicating *Najas flexilis* condition.

3.5 Conclusion

SWT is a much better predictor of the condition of a lake than NVC due to the greater number of species included, this means it can detect finer scale differences in the underlying environment. However, due to the narrow niche that *Najas flexilis* inhabits a further division in SWT 5 is required. These SWTs could indicate the at risk sites for *Najas flexilis* and would include sites at the most eutrophic and oligotrophic ends of the *Najas flexilis* range. These would be lakes that contain SWT 3, and the more eutrophic SWT 5 communities. If SWT were used as a preliminary monitoring regime, perhaps in conjunction with water and sediment chemistry information (see Chapter 2), more in depth monitoring efforts concentrating on *Najas flexilis* itself could be targeted at at-risk lakes supporting SWT 5 and 3. This would save time and resources even though some of these lakes would contain healthy populations.

Summary

- The lake scale qualitative approach elucidated significant differences in pH, alkalinity and the concentration of total oxidised nitrogen and dissolved reactive phosphate in the water as well as differences in phosphate, calcium and iron in the sediment.
- The small-scale quantitative approach only elucidated a difference in light extinction coefficient between community groups.
- The community groups attained from the small-scale quantitative survey were roughly comparable to NVC communities A13, A24a and A23.
- The lake scale qualitative approach produced community groups comparable to standing water type 3, 4 and 5, however, this study produced two type 5 sub-groups.
- Extinct *Najas flexilis* sites were classified as type 3 and the most eutrophic type 5, as were other sites that still supported *Najas flexilis*.

4. A trait based methodology to predict competition between native and invasive species: *Najas flexilis* and *Elodea* spp.

4.1 Introduction

4.1.1 Aims

- To determine whether the incidence of individual, or sets of, morphological, physiological and/or life-history traits exhibited by the associated species forming lake *Najas flexilis* communities can act as an indicator of the degree of probable competitive impact on *Najas flexilis* fitness in such habitats
- To use this information to assess the likely competitive threat posed by *Elodea canadensis* and *Elodea nuttallii* to *Najas flexilis* survival in Scottish lakes.

4.1.2 Background

The previous chapters have concentrated on the relationships between *Najas flexilis* growth and the physical and chemical environment of the lake habitat. The composition of macrophyte communities has been considered as a method to describe this environment, and assess the suitability of the environment for *Najas flexilis* growth. However, associated aquatic plant species may have a more direct impact on *Najas flexilis*. Competitive interactions, coexistence and coevolution are all factors that may play an important role in determining the distribution and abundance of plant species (Rosch *et al.*, 1997).

4.1.2.1. *Elodea* spp. in Scotland

Elodea canadensis and *Elodea nuttallii* are native to North America, yet behave as invasive aquatic weeds throughout Europe. They have also caused problems in Japan, Australia and New Zealand (Murphy *et al.*, 1990; Preston & Croft, 1997). *Elodea* spp. appear to exhibit a series of successional growth phases after invasion of a new site. After introduction to a new habitat, *Elodea canadensis* often grows in abundance to nuisance levels, over a period varying from a few months to 4 years (Phase 1). The plant may then persist at high abundance for as many as 5 years during the second phase, and then wanes either gradually or quickly during the third phase. In the fourth phase *Elodea canadensis* persists at non-nuisance levels (Sculthorpe, 1967). If a disturbance such as cutting interrupts these phases, the process can start again. *Elodea nuttallii* biomass has also been reported to naturally

fluctuate and after an initial period of infestation has been seen to decline of its own accord (Hamabata, 1997; Simpson, 1990).

Table 4.1 Sites in Scotland where *Najas flexilis* and *Elodea* spp. occurred together during 1998-2000

Site	Grid reference	Area	*Elodea canadensis*	*Elodea muttallii*
Loch of Butterstone	NO 062453	Perthshire	✓	✓
Loch of Craiglush	NO 044446	Perthshire	✓	✓
Loch of Lowes	NO 055443	Perthshire	✓	✓
Loch Clunie	NO 116444	Perthshire	✓	✓
Lake of Menteith	NS 577002	Stirling	✓	✓
Tangy Loch	NR 695280	Kintyre	✓	×
Loch Nan Gad	NR 783573	Kintyre	✓	×
Loch Fada	NR 383955	Colonsay	✓	×
Loch Scarie	NF 718705	North Uist	×	✓
Loch Grogary	NF 717710	North Uist	×	✓
Loch nam Faoileann	NF 752210	South Uist	✓	×

The majority of the mainland lakes in Scotland containing *Najas flexilis* also contain one or both of the *Elodea* spp. (Table 4.1). The invasion of *Elodea* spp. into these sites does not appear to have been detrimental to *Najas flexilis*, and the *Elodea* spp. exist in small quantities within limited areas of the lakes concerned. It could be that *Elodea* has already invaded, had its initial abundance phase and now exists in a small quantities or it could be that it has not reached its abundance phase or it may never happen. With one exception, these mainland lakes are at the larger end of the scale of those inhabited by *Najas flexilis* in Scotland and as such can be presumed to be environmentally more heterogeneous than the smaller, shallow lakes of Uist and Colonsay. During the initial *Elodea* spp. invasion, this environmental heterogeneity may have ensured *Najas flexilis* survival, due to the occurrence of refugia where *Najas flexilis* could successfully compete with the *Elodea* spp. or where *Elodea* spp. could not grow. Tangy Loch (Kintyre) is the exception among the mainland sites. This is a smaller lake, where *Elodea canadensis* is a recent invasive. In 1994, *Elodea canadensis* was extremely abundant and a specific diver search for *Najas flexilis* at this time only found two plants within the loch (Martin Gaywood, Scottish Natural Heritage, pers. comm). Whether the invasion of *Elodea* spp. caused the extinction of *N. flexilis* in sites where *N. flexilis* can no longer be found is unknown. Both *Elodea* spp. can be found in some, but not all of these 'absent' sites. However, the environment at these absent sites also appears unfavourable for *N. flexilis* growth (Chapter 2). Therefore reintroduction without environmental improvement is not an option.

One of the major threats to the *Najas flexilis* stronghold in the Hebrides is weed competition, specifically from *Elodea canadensis* and *Elodea nuttallii*. This is because in Colonsay, and North and South Uist the threat of pollution and eutrophication is small, due to the lack of intensive agriculture or any other kind of industry. However, there have been a series of introductions of alien species, including the two aforementioned aquatic weed species.The Scottish Natural Heritage (SNH) Fresh Water Loch Survey Team first reported *Elodea canadensis* in Loch nam Faoileann in S. Uist, near Daliburgh, in August 1995 (Scott, 1996). *Elodea nuttallii* is also now present in North Uist in Lochs Grogary and Scarie. This is a recent introduction, probably occurring in the early 1990's although no hard data are available. This invasion in Loch Grogary had reached a state where it was restricting boat use by anglers in 1999. These recent invasions are a cause for concern for the conservation agencies because as well as being the stronghold for *Najas flexilis* in Britain, the Uist lochs support rare *Potamogetons* such as *Potamogeton rutilus* and *Potamogeton epihydrus,* as well as other uncommon plants such as *Callitriche hermaphroditica.* The N. Uist lochs where *Elodea nuttallii* has invaded are two of only 17 known current sites for *Potamogeton rutilus* in the British Isles.

North and South Uist have many lochs often connected by small streams and drainage ditches (Waterston *et al.*, 1979). This intricate network of freshwater bodies is ideal for the spread of *Elodea* spp., which rely on the movement of vegetative fragments and turions for dispersal in the UK. The popularity of angling in Uist and the movement of fishermen from loch to loch is a vector for *Elodea* spp. dispersal, a single viable fragment transferred in fishing tackle could establish a new population in another loch. This fear was to some extent realised when SNH commissioned a survey of Loch nam Faoileann and the surrounding lochs for *Elodea canadensis* in October 1996. This found that *Elodea canadensis* was now in four lochs in the vicinity rather than one, as previously thought (Scott, 1996).

The concern of the conservation agencies is that as the *Elodea* spp. spread and invade new lochs and lochans in Uist, either of the *Elodea* spp. at the height of their abundance could outcompete *Najas flexilis*. This is only a threat if competition actually occurs between these species (and no habitats exist in which *Najas flexilis* can successfully withstand competition from *Elodea* spp.) and if *Najas flexilis* does not create a persistent seedbank. There is little knowledge on *Najas flexilis* seedbank persistence and the length of time required to carry out experiments into this exceeds the duration of this study.

4.1.3 Elodea spp. vs. *Najas flexilis*

4.1.3.1 Life history traits

Reproduction, perennation and regeneration

Najas flexilis is a monoecious, hydrophilous, annual, which cannot reproduce vegetatively, consequently it relies exclusively on seed for perennation and dispersal (Haynes, 1979; Preston & Croft, 1997; Triest, 1988). Germination begins when there is sufficient light and warmth. Haas (1998) believes this is at a temperature of around 19°C in the second half of June, in the Swiss Alps. However, the time of year at which *Najas flexilis* begins to germinate in Scotland appears to be the beginning of June, (James, personal communication) although plants are not big enough to be caught in a grapnel until July (personal observation). A lower temperature than 19°C probably prompts germination in Scotland. Experiments conducted by Hay and Wingfield on seeds collected from Scottish lochs found seeds could germinate at 11°C (section 6.3.1). Germination is also probably prompted by extended day length. Experiments also showed that greater germination occurred in light rather than dark conditions (section 6.3.1).

Both *Elodea* spp. are dioecious, epihydrophilous, perennials (Cook, 1996; Spicer & Catling, 1988), however only female plants of both species have been reported in Britain in recent times (the last time a male flower was reported was on *Elodea canadensis* in 1903). Therefore, in Britain reproduction by these two species is entirely by vegetative means. This is chiefly by undifferentiated vegetative fragments, although unspecialised turions are produced by *Elodea canadensis* in the autumn (Preston & Croft, 1997). Plants of *Elodea canadensis* die down in the winter, regrowing from underground stems and turions in the spring. *Elodea nuttallii* overwinters as prostrate shoots at the bottom of the water, new shoots grow upwards in the spring, elongating rapidly (Preston & Croft, 1997). Kuni (1982) found that *Elodea nuttallii* grew in winter at temperatures above 4°C and that plants grew actively when water temperatures were higher than 10°C. No such information is available for *Elodea canadensis.* This suggests that *Elodea nuttallii* would have a competitive advantage over *Najas flexilis* by being able to grow at cooler temperatures earlier in the year.

4.1.3.2 Growth

Both *Najas flexilis* and *Elodea* spp. exhibit an elodeid life form, however, *Elodea* spp. grow

to the water surface in order to flower. *Elodea nuttallii* can grow to heights up to 1.5m (Simpson, 1990) and produce canopies at the surface while *Najas flexilis* grows only to a height of 30 cm (personal observation).

Elodea canadensis possesses a Competitive Ruderal (CR) strategy (Grime *et al.*, 1988). This means the species is both a good competitor and grows well in disturbed habitats. This is a copetite*Elodea nuttallii* is thought to be more aggressive than *Elodea canadensis* and grows more frequently in the nutrient rich sites rather than the more mesotrophic sites where *Elodea canadensis* is found (Grime *et al.*, 1988; Murphy *et al.*, 1990; Preston & Croft 1997) and is therefore probably also a CR strategist. *Najas flexilis* has not undergone the required studies in order to assign a strategy to it, however, its annual life style and seed production along with comments in the literature (see section 2.1.5) suggests that it is a disturbance tolerator (as are most other *Najas* species (Murphy *et al.*, 1990). The ability of the plant to withstand low light levels (Hough & Fornwall, 1988) suggests that its strategy is also likely to contain an element of stress tolerance. Therefore, from evidence in the literature it appears to be likely that *Najas flexilis* possess a Stress tolerant Ruderal (SR) life strategy.

Although both species appear to possess ruderal traits, the type of disturbance tolerated by *Elodea* spp. and *Najas flexilis* is quite different. *Najas flexilis* colonises freshly disturbed sites (section 2.1.4 & 2.1.5; Hagley *et al.*, 1996; Pearsall, 1920; Tazik *et al.*, 1982). However, disturbance of *Najas flexilis* during the growth season is of no benefit to the species, vegetative fragments produced by disturbance will not grow (personal observation). Once seeds have been produced disturbance, often in the form of autumnal gales, will break up the plant and disperse seeds attached to plant fragments throughout the water body. These fragments do not float readily, however they often reach the shoreline of lakes (see section 5.1.3.3 for role of disturbances in dispersal). The seeds produced enable *Najas flexilis* to survive disturbance and stress (such as ice formation overwinter) permitting population regrowth the following spring. Therefore, disturbance is important for the species at certain times within its life cycle yet at others can be detrimental, hence the relationship between exposure and depth for *Najas flexilis* (section 2.3.4).

Elodea spp. also take advantage of freshly disturbed areas and colonise open spaces like *Najas flexilis*, although unlike *Najas flexilis*, disturbance within the growth phase of *Elodea* spp. is not detrimental and can even aid dispersal. Both *Elodea* spp. tolerate a greater

amount of disturbance than *Najas flexilis* (*Najas flexilis* is limited to lakes, where as *Elodea* spp. will also grow in slow flowing rivers). Disturbance such as mechanical cutting of *Elodea* spp. can lead to their proliferation, as the separate fragments produced by the disturbance are all viable disseminules (Abernethy *et al.*, 1996; Grime *et al.*, 1988; Murphy *et al.*, 1990; Preston & Croft 1997; Sculthorpe, 1967). This tolerance of disturbance within the growth phase of the life cycle is in contrast to the tolerance of disturbance by *Najas flexilis.*

As discussed previously (section 2.1.3) *Najas flexilis* cannot withstand high pH and low CO_2 conditions (Hough & Fornwall, 1988; Hough & Wetzel, 1978; Wetzel & McGregor, 1968), and this is a possible contributory factor to its decline in Scotland. In such conditions, the plant does not appear to immediately die, but just fails to grow further until conditions are more favourable (This is a personal observation in experimental conditions only). *Elodea nuttallii* and *Elodea canadensis* in contrast can utilise HCO_3^- (James *et al.*, 1999). In slow flowing or still waters, it is quite usual to have CO_2 limited conditions in times of high photosynthesis by either algae or macrophytes, due to their high consumption of this compound for photosynthesis (Jones *et al.*, 1996). For *Najas flexilis,* this would mean that metabolism and growth would come to a halt. *Elodea nuttallii* appears to be able to utilise HCO_3^- within 5 days of exposure to a low CO_2 concentration and can continue metabolism and therefore growth (Jones *et al.*, 1993). This continued metabolism would suggest a competitive advantage over *Najas flexilis.* This will only be a problem in systems that are not limited in other ways by light or nutrients.

4.1.3.3 Environment

This differential ability to utilise HCO_3^- begins to explain the differences in the environments in which these species occur. Both *Elodea* spp. can inhabit more eutrophic sites than *Najas flexilis,* with *Elodea nuttallii* capable of inhabiting the most eutrophic sites of all three species (Palmer, 1989; Preston & Croft, 1997). Whether *Elodea nuttallii* can colonise the nutrient poor sites that *Najas flexilis* can inhabit in Britain is as yet unknown. It seems likely that it will compete less efficiently in such conditions due to its preference for more eutrophic sites. *Elodea* spp. have previously been reported to be limited by low nitrogen levels elsewhere in Europe (Best *et al.*, 1996; Madsen *et al.*, 1998).

All three species can grow at depths up to 3 m although they are more commonly found in

shallower water (Simpson, 1990). *Najas flexilis* has been found in extreme depths; 9 m in Lake George, USA (Sheldon & Boylen, 1975) and 14m in Shoal Lake, Manitoba-Ontario (Pip & Simmons, 1986). *Elodea* spp. appear to be found more frequently in shallower water than *Najas flexilis* (personal observation), most probably due to its slightly better resistance to wave exposure and better competitive abilities to compete with other plants utilising the greater quantities of light. Neither species appear to grow in the shallow littoral zone of gently sloping lakes, most commonly dominated (in Europe) by *Littorella uniflora* communities.

When the species all occur in the same lake, they do so in similar areas of that lake. The macrophyte surveys described in section 3.2.2.2 found *Elodea canadensis* in the *Najas flexilis* micro-habitat in 6 out of 7 lakes where both occurred, whilst *Elodea nuttallii* was found in the *Najas flexilis* micro-habitat in 2 out of 5 lakes where both occurred. This illustrates that *Elodea* spp. and *Najas flexilis* can and do cohabit the same habitats. This niche overlap, suggests there is a strong likelihood of competition. *Elodea* spp. also inhabit environments where *Najas flexilis* does not grow i.e. in eutrophic and slow flowing systems. However, whether *Najas flexilis* grows in environments uninhabitable to the *Elodea* spp. is unclear; if this is the case, it appears that they would most likely be in low light and nutrient poor conditions.

The comparison of life history strategies suggests that *Elodea* spp. may well have the competitive advantage over *Najas flexilis* due to its ability to grow earlier in the growth season and to produce canopies, suggesting competition for light may be a problem for *Najas flexilis*. The ability of both *Elodea* spp. to utilise bicarbonate also suggests they would have a competitive advantage in conditions of sufficient light and low dissolved carbon dioxide concentration. Whether these traits will actually confer a competitive advantage over *Najas flexilis* in the environments where *Najas flexilis* grows has not hitherto been determined.

Whilst experiments provide valuable information on competition during the established phase of plant growth, such studies on aquatics do not always appear to find any evidence of competition or of strict competitive hierarchies, or the hierarchies doe not concur with what is observed in the field. It has been suggested that this may be due to competition occurring outwith this stage i.e. occurring in the establishment stage (Chambers & Prepas, 1990; Greulich & Bornette, 1999; McCreary, 1991; Spencer & Ksander, 2000). *In situ*

experiments on the establishment phase are difficult to conduct, as the diaspores are difficult to track. In aquatic systems, these problems are exacerbated, as the importance of different types of diaspores for each species is unknown (i.e. whether colonisation is mainly via turions, vegetative fragments, seeds, rhizomes or tubers).

Results that can be directly transferable to the natural system in which plants grow can also be difficult to achieve if the levels of stress and disturbance experienced by the plants in natural systems is unknown. *In situ* experiments experience realistic levels of stress and disturbance, however plants that are damaged by disturbance during the experiment are often excluded from the results (Greulich & Bornette, 1999) when their ability to tolerate disturbance will effect the plant community composition.

These problems associated with controlled experimental studies on establishing macrophyte population interactions are the reasons why I adopted a community trait based approach in this study. By looking at which traits conferred a competitive advantage of associate species over *Najas flexilis* in the natural communities and environments where *Najas flexilis* grew, all phases of plant growth were considered, as there was no artificial start date, plant or population size. The environmental stress and disturbance were real, not simulated, and damage by disturbance before the sample date would not be excluded.

The relationship between the traits of an organism and habitat characteristics have been studied by a number of authors working in different habitat conditions (e.g. Grime *et al.*, 1988; Willby, 2000; Lavorel *et al.*, 1997) and some authors have used such knowledge to predict aquatic community composition (see Murphy, 1990; Greulich, 1999). Traits have also been used to look at the success of alien invasions (Pysek *et al.*, 1995). The aim of my study was to determine if *Elodea* spp. possessed a strategy (i.e. "collection of traits": Grime *et al.*, 1988) which would be more successful than the strategy possessed by *Najas flexilis* across the environmental conditions applying to *Najas flexilis* habitats in Scotland, so that it could be determined how big a threat the *Elodea* spp. are to *Najas flexilis*. Intrinsic to Grime's strategy theory is the fact that the species possessing the 'competitor' strategies will not be the most successful species in all environments i.e. if the environment is highly disturbed or stressed (Grace, 1990). Therefore, in experimental productive non-stressful environments with no disturbance *Elodea* spp. may well be the 'competitor' species. However, if the conditions in which *Najas flexilis* grows are stressful and disturbed rather than productive, *Najas flexilis* may be the more successful species. Comparison of the traits

of the plants which do have a competitive effect on *Najas flexilis,* in the environment in which it grows, to those possessed by *Elodea* spp. may provide a useful insight into the ability of *Elodea* spp. to compete with *Najas flexilis* in its native Scottish habitats.

4.2 Methods

Leaf area/shoot length was selected as a measure of plant fitness, as an indicator of plant success for this analysis. This is because the components which competitor species are most likely to compete for are nutrients, light and CO_2. Competition for nutrients is often shown by an increased size of nutrient foraging organs such as the roots. Some aquatics may utilise nutrients from the water as well as the sediment, however *Najas flexilis* has been found to use phosphorus predominantly from the sediment (Carignan & Kalf, 1980). Unfortunately, the root structures of *Najas flexilis* are very fragile and there is not sufficient confidence that the roots collected represented the whole root system of *Najas flexilis*, therefore this cannot be used as a trait illustrating the fitness *of Najas flexilis.* Light has been shown to affect leaf area/shoot length rather than the number of reproductive structures/shoot length (see models in section 2.3.2.2). In good growth conditions, the high levels of photosynthesis can reduce CO_2 availability; this is represented by an increase in pH. As Fig 2.1 and 2.2 illustrates the decrease in *Najas flexilis* fitness at high pH is due mostly to a decrease in leaf area/shoot length, although this does have knock on effects for reproductive number as the reproductive structures only grow in the leaf nodes. As the purpose of this investigation is to discover which plant traits are most likely to affect *Najas flexilis*, and not to use the consequent models to predict the success of *Najas flexilis* at various sites, leaf area/shoot length is the fitness variable used, as that is the variable most likely to show a response to competition.

In order to obtain quantitative measures of plant traits for the species growing in association with *Najas flexilis*, quantitative abundance measures of the plant species growing in association with *Najas flexilis* were obtained. These were taken using Ekman grabs (three per site), the mean dry weight of the plant species collected in the Ekman grab was then used as the quantitative abundance measure (see section 3.2.2.2). This data was then used to produce a quantitative site x species matrix. This was carried out twice, once including and once excluding the *Najas flexilis* plants collected in the Ekman grabs. This was done so that inter-specific competition could be looked at separately to total competition (intra and

inter-specific competition). This is because the potential competitor species will only contribute to inter-specific competition.

The matrix of the attributes possessed by European hydrophytes produced by Willby *et al.* (2000) was used as the attribute matrix in this study. This matrix includes traits on leaf morphology, growth form and reproduction and perennation characteristics. The full list of traits is in Table 4.2. A trait such as leaf area is split into four attribute groups; small, medium, large and extra large (defined in Table 4.2). Each species is then scored 0 if the attribute is absent, 2 if present and 1 indicating occasionally, but not generally exhibited. This species x attribute matrix was produced by (Willby *et al.*, 2000) from the literature and extensive fieldwork experience with European hydrophytes and was therefore not specific to particular environmental conditions, and it contained all the species found in association with *Najas flexilis*. It was therefore deemed possible to use the matrix directly in this study.

Table 4.2 Traits and attributes investigated, from (Willby *et al.*, 2000). Morphology index = (plant height + lateral extension of the canopy)/2

Trait	Attribute
Growth form	Free-floating, surface
	Free floating, submerged
	Anchored, floating leaves
	Anchored, submerged leaves
	Anchored, emergent leaves
	Anchored heterophylly
Vertical shoot architecture	Single apical growth point
	Single basal growth point
	Multiple apical growth point
Leaf type	Tubular
	Capillary
	Entire
Leaf area	Small (<1cm^2)
	Medium (1-20 cm^2)
	Large (20-100 cm^2)
	Extra large (>100 cm^2)
Morphology index (score)	2
	3-5
	6-7
	8-9
	10
Rooting at nodes	-
High below-ground:above ground biomass	-
Mode of reproduction	Rhizome
	Fragmentation
	Budding
	Turions
	Stolons

	Tubers
	Seeds
Number of reproductive organs year^{-1} individual^{-1}	Low (<10)
	Medium (10-100)
	High (100-1000)
	Very high (>1000)
Perennation	Annual
	Biennial/short lived
	Perennial
Trait	Attribute
Evergreen leaf	-
Amphibious	-
Gamete vector	Wind
	Water
	Air bubble
	Insect
	Self
Body flexibility	Low (<45 °)
	Intermediate (45-300°)
	High (>300°)
Leaf texture	Soft
	Rigid
	Waxy
	Non-waxy
Period of production of reproductive organ	Early (March-May)
	Mid (June-July)
	Late (August-September)
	Very late (post September)
Fruit Size	<1mm
	1-3mm
	>3mm

This matrix did not include functional attributes such as growth rate or the ability to utilise bicarbonate, therefore these functional traits were not included in this study. CSR strategy is not included in the trait types either as many macrophyte species have not undergone the required studies for strategy allocation. This is a limitation in this approach as sufficient functional information (as opposed to trait or attribute information where the specific function may not be known) is not available for the majority of aquatic macrophytes found in Britain.

The species abundance x site matrix was then multiplied by the species x attribute matrix so that a quantitative measure of attribute abundance at each site was produced. For example, the abundance of species 1 at site 1 was multiplied by the score given to species 1 for attribute 1. The abundance of species 2 at site 1 multiplied by the score given to species 2

for attribute 1 was added to this number and so forth, until all the species abundances for site one had been multiplied by their attribute scores for attribute 1 and summed together. If attribute 1 was medium leaf area, you would then gain the total abundance of medium sized leaves at site 1. This is then repeated for each site and each attribute until an attribute abundance x site matrix is produced. This was carried out twice, once using the species abundance x site matrix including *Najas flexilis* and once using the species abundance x site matrix excluding *Najas flexilis.*

The abundance of a particular attribute at the range of sites was then regressed against the fitness measure, leaf area/shoot length, at those sites. This gives an indication of the attributes whose abundance in a community has an effect on *Najas flexilis* fitness. Stepwise regression was then used to create a multiple regression model predicting leaf area /shoot length from the abundance of attributes. Those traits showing a negative relationship with *Najas flexilis* fitness were then compared to the traits possessed by *Elodea* spp. to see if they possessed the necessary traits to be a threat to *Najas flexilis.* Such knowledge could also be used if new species were to become invasive in Britain, if the new invasive species possessed the 'competitive traits' it would be known that there was a potential problem for *Najas flexilis.*

4.3 Results

4.3.1 Inter specific competition

Regressions between *Najas flexilis* fitness measure leaf area/ shoot length and the abundance of traits in the plant community excluding *Najas flexilis* attributes, showed that the none of the attributes had a significant relationship ($p < 0.05$) with *Najas flexilis* fitness. This illustrates that the presence of a single trait in the community does not explain the variation in *Najas flexilis* fitness well. However, comparing the most significant results ($p<0.1$) for interspecific and total competition does illustrate the effect of including *Najas flexilis* as a potential competitor with itself. Those attributes that had the most significant relationship ($p<0.1$) with *Najas flexilis* fitness are shown in Table 4.3.

4.3.2 Total competition

When the abundance of attributes in the entire plant community, including *Najas flexilis,* were regressed with *Najas flexilis* fitness, the only attributes where $p<0.1$ were those attributes which were not effected by the inclusion of *Najas flexilis* attributes. These

attributes were the quantity of perennials, wind pollinators and plants that flowered in June-July; *Najas flexilis* does not posses any of these traits. The quantity of seed producers and anchored submerged leaved plants within the plant community was increased by the inclusion of *Najas flexilis* plants and the relationship was no longer significant. The attributes which had a less significant relationship with *Najas flexilis* fitness ($p>0.1$), when the abundance of traits possessed by plants in the community, excluding *Najas flexilis,* was used in the regression analysis, did not have a more significant relationship with *Najas flexilis* fitness, when the abundance of traits possessed by plants in the community, including *Najas flexilis,* was used in the regression. This suggests that interspecific competition is more important when predicting *Najas flexilis* fitness than total competition.

Table 4.3 Results of the regression analysis between attributes of the plant community (excluding *Najas flexilis*) and *Najas flexilis* fitness measure; leaf area/ shoot length, where $P < 0.1$. Attributes with an * are those attributes where $p < 0.1$ when *Najas flexilis* plants are included in the plant community trait abundances.

Attribute	Relationship	r^2 value	P value
Perennial*	Quadratic	0.183	0.088
Anchored submerged leaves	Quadratic	0.19	0.080
Seed production	Quadratic	0.179	0.093
Wind pollination*	Quadratic	0.187	0.084
Flowering June-July*	Quadratic	0.203	0.066

In contrast to single traits in the community, the combined traits do have a significant relationship with *Najas flexilis* (Model 1). The combined traits that explained the most variation in the *Najas flexilis* fitness were flowering in June-July, wind pollination and being a perennial. Therefore, the best equation for predicting total competition and interspecific competition is the same:

Model 1: $r^2 = 0.686$ $p = 0.031$ $n = 27$

$La/sl = 0.187 + 2.987FJJ - 12.267FJJ^2 + 1.582WP - 0.769WP^2 - 3.656P + 10.99P^2$

Where:

La/sl = Leaf area/shoot length

FJJ = Flowers in June-July

WP = Wind pollinated

P = Perennial

Table 4.4 A comparison of the attributes possessed by both *Elodea* spp. and those with the most significant ($p < 0.1$) relationship with *Najas flexilis* fitness. ✓= possession of a trait, ✖ = absence of a trait.

Attribute	*Elodea canadensis*	*Elodea nuttallii*
Perennial	✓	✓
Anchored submerged leaves	✓	✓
Seed production	✖	✖
Wind pollination	✖	✖
Flowering June-July	✓	✓

Table 4.5 European hydrophyte species investigated in (Willby *et al.*, 2000) which regularly exhibit all the attributes with the most significant relationship with *Najas flexilis* fitness where $p < 0.1$ and also grow in habitats with permanent standing water with a depth of over 0.5 m.

Species
Callitriche hamulata
Juncus bulbosus
Littorella uniflora
Myriophyllum alterniflorum
Potamogeton alpinus
Potamogeton gramineus
Potamogeton lucens
Potamogeton natans
Potamogeton perfoliatus
Potamogeton praelongus
Schoenoplectus lacustris
Sparganium emersum

The *Elodea* species do possess some of the attributes which have a relationship with *Najas flexilis* fitness where $p<0.1$, but not all of them (Table 4.4). There are 13 species native to

the British Isles[1] that grow in the same environment as *Najas flexilis*, which possess all these attributes (Table 4.5). These species have all been seen growing in lakes containing *Najas flexilis,* see Table 3.3, although not all have been found in the Ekman grab samples used to collect quantitative macrophyte samples within the micro-habitat where *Najas flexilis* grows. This may be due to chance or could be due to environmental factors. To be included in this list, the species had to grow in permanent standing water at a depth of greater than 0.5m. The trophic state of the waterbodies in which these species grow, was not a determining factor for inclusion in this list. This is because *Najas flexilis* is characteristic of mesotrophic lakes. Such lakes can contain some predominately eutrophic species such as *Elodea nuttallii* and some predominately oligotrophic species such as *Lobelia dortmanna*[2]. Therefore, in order not to eliminate any species from this list unnecessarily only the aforementioned three obvious physical parameters were applied as prerequisites to inclusion in this list.

4.4 Discussion

This approach does not indicate the reason why increased quantities of perennial, wind pollinated, early flowering plants have a quadratic relationship with *Najas flexilis* fitness. There are three possible reasons for this relationship: 1) The increased presence of these plant traits are merely indicators of environmental conditions unsuitable for *Najas flexilis* growth and have no direct or indirect effect on *Najas flexilis* fitness. 2) The increased presence of the plant traits may represent the occurrence of 'apparent' competition as described by Connell (1990) where there is an indirect interaction via a shared enemy or other species on the same trophic level. 3) The relationship between the plant traits and *Najas flexilis* fitness may represent 'real' competition; where there is a direct interaction, where the plants with these plant traits interfere with *Najas flexilis*, or an indirect interaction, where both plants containing these plant traits and *Najas flexilis* compete to exploit a shared resource.

Using this approach it is impossible to determine whether the abundance of these traits are indicative of a certain environmental condition which is unsuitable for *Najas flexilis* fitness

[1] The species list used by Willby (2000) was used to determine which species possessed the appropriate traits and grew in the appropriate environment as this was a consistent source of information euhydrophyte attributes and their habitats Therefore species not found in the species list in Willby (2000) will not be found in the Table 4.5

[2] The aforementioned species are classified as eutrophic or oligotrophic according to Willby (2001) and Palmer's DOME code (Palmer, 1989)

or whether they are actually causing the deleterious effect. For example, in section 2.3.2.2 it was seen that total phosphate levels in the water had a relationship with *Najas flexilis* fitness. However, it is unlikely but possible that high phosphate levels alone affect *Najas flexilis* fitness. It is more likely that the high phosphate levels are leading to increased production by photosynthetic organisms, this may well lead to direct competition for light or indirect competition for a resource such as carbon dioxide. This illustrates one of the drawbacks of this approach. Experiments would be able to elucidate the response of *Najas flexilis* to an increase in total phosphate with and without macrophyte competition, however it would be difficult to conduct experiments with excess phosphate in the water without a limited amount of algae growing in the water and invalidating the experiments. This condition of increased phosphate availability without an increase in photosynthetic organisms is unlikely to occur in real systems unless another resource essential for growth is limited. However, it should be considered that the abundance of these attributes is a response to the environment.

Apparent and real competition are equally difficult to tease apart both in experimental and observational studies such as this. The quadratic relationship would fit with both the hypothesis of real and apparent competition. In the case of real competition the downslope of the curve can be explained by the fact that at high levels of plant growth, *Najas flexilis* has a reduced fitness due to competition for light or another resource. However there is also an apparent positive density dependence (the upslope of the curve) when competitor species abundance is low. One possible reason for positive density dependence is that of exposure. It has already been discussed that *Najas flexilis* is a fragile plant and how in lakes with a large fetch it is limited to water of greater depth because of this (section 2.3.4). It has been shown that water movement is often slowed by plant growth (Losee & Wetzel, 1993). Therefore in situations where *Najas flexilis* has no immediate neighbours to reduce exposure, it could perform less well than when exposure is limited to a certain extent by macrophyte growth, but is not yet experiencing competition greater than this advantageous effect. This may be particularly important at the seedling establishment phase, when plants have not yet developed sufficient anchorage. Alternatively, it could be that in environments not suited for other macrophyte growth, *Najas flexilis* also grows below its optimum.

4.4.1 Flowering time

The quantity of vegetation that flowered between June and July had a relationship with *Najas flexilis* fitness. It is of note that more species found in close association with *Najas flexilis* flowered in August-September rather than June-July, but that trait did not affect *Najas flexilis*. This early flowering time is indicative of early growth in contrast to *Najas flexilis,* which does not germinate until June. This is important, as it will mean that as *Najas flexilis* germinates and begins to grow other species will have already intercepted the light and could have depleted nutrient resources, or already have developed greater root and shoot sizes to effectively forage for resources such as nutrients and light. This will not only affect *Najas flexilis* ability to grow but also the amount of resources available to be allocated to seeds for the next generation. Therefore, this early development is important for competition and has been found to be so in many systems many times (Grime *et al.*, 1988; Pachepsky *et al.*, 2001; Spencer & Ksander, 2000). It is also at this establishment stage where most competition in aquatics has been found to occur (McCreary, 1991).

4.4.2 Perennation

If competition, real or apparent, occurs it seems important that the competitor species are perennial. A perennial nature would allow these plants to start to grow quicker after conditions become suitable for growth, this is because more resources will be available to the plant if it has overwintered as tubers, or rhizomes, than as a tiny seed like *Najas flexilis*. If the perennial plant has overwintered as vegetative fragments like *Elodea* spp. the photosynthetic organs are already in existence and active growth can begin the moment conditions are suitable, rather than first having to produce photosynthetic organs as would be the case, if first the plant has to germinate from a seed, like *Najas flexilis.* This may begin to explain the relationship between disturbance and *Najas flexilis.* Disturbance can remove perennials, leaving the habitat open and free of any competition from earlier developing macrophytes, so that there is no competition when *Najas flexilis* emerges later in the season. The method of perennation does not seem to confer any particular advantage whether it is by rhizome, fragmentation, budding, turions, stolons, tubers or evergreen existence.

4.4.3 Seed production

In contrast to perennation mechanism, seed production does have a relationship with *Najas flexilis,* although this does not indicate whether seed production regularly leads to

germination and growth except in annual species (annual species do not have a relationship with *Najas flexilis* fitness). The benefit of being a seed producing perennial would be that the seeds could colonise the newly disturbed bare patches, and then in subsequent springs it would be quicker to grow from perennating organs and such plants could then successfully compete with *Najas flexilis*. However, turions or vegetative fragments may be as mobile and effective as some seeds at establishing new populations.

4.4.4 Wind pollination

The abundance of wind pollination in the community also affects *Najas flexilis* success. This could be for one of two reasons. Firstly, it could be because wind pollination is more successful and reliable than any other form, and leads to the production of more progeny, and due to this increased number of progeny the plant is more competitive. This would assume that recruitment via seed was an important aspect of the community dynamics and that wind pollination is the most effective pollination mechanism in macrophytes. Although it has been suggested that hydrophilous pollination may not be effective there is little evidence to confirm or refute this (Sculthorpe, 1967). There is observational evidence that reproduction from seed is not abundant, but it cannot be excluded as a possibility as it is presently not perceived as probable, as there is little experimental evidence to support this view.

A second hypothesis as to why wind pollination is important is that those species involved in wind pollination must position their flowers upon the water surface. This process often involves growth up towards the water surface, and these taller plants will be likely to exhibit a shading effect over the shorter plants, the floating flowers may themselves also exhibit a shading effect. Other pollination types will also exhibit this effect; this includes epihydrophilous pollination and non-submerged insect pollination. Epihydrophily (water pollination at the water surface) and hyphydrophily (under water pollination) (Faegri & van der Pijl, 1979) were not differentiated for the purpose of this study. Therefore, it is not surprising that once combined hydrophily had no significant relationship with *Najas flexilis* success. Insect pollinated plants could show a similar competition effect to wind pollinated plants due to the fact that both types of plants must grow to the surface to support flowers above the water and that flowers and stems and leaves growing towards the surface would potentially shade competitors. An example of aquatic entomophilous plants exhibiting these traits is the water lilies; *Nymphaea alba* and *Nuphar lutea*. Although these species were

often found in lakes containing *Najas flexilis,* they were never found in close association with them. The only insect pollinated species found in the *Najas flexilis* micro-habitat were *Utricularia* spp. and *Lobelia dortmanna,* both of which were only occasionally found in this micro-habitat. It is therefore no surprise that no relationship could be detected between insect pollination and *Najas flexilis* fitness.

4.4.5 Submerged aquatic leaves

All the plants growing in close association with *Najas flexilis* possessed submerged aquatic leaves. This is most likely because of the deep water environment in which it grows. Plants whose leaves could also be emergent such as *Littorella uniflora* and *Juncus bulbosus* also grew in association with *Najas flexilis,* yet were never seen to emerge from the water in the *Najas flexilis* micro-habitat. Plants which also possessed floating leaves such as *Potamogeton gramineus* and *Sparganium angustifolium* did grow in close association with *Najas flexilis* and their floating leaves were observed, however they never dominated the community. This is probably why only the anchored submerged leaves had a relationship with *Najas flexilis* fitness. The lack of a species with floating leaves dominating the community could be due to the environment in which *Najas flexilis* grows being unsuitable for their growth. Alternatively floating leaved plants could be such effective competitors that they exclude *Najas flexilis* growth. Certainly floating leaved plants such as *Potamogeton natans* often grew contiguous to, but not intermingled with, *Najas flexilis.* This occurred for example at Loch a' Bhada Dhraich, where *Najas flexilis* only grew in this situation with *Potamogeton berchtoldii.*

4.4.6 *Elodea* spp. as a competitor with *Najas flexilis*

Elodea spp. possess some but not all of the traits discussed above as having a relationship with *Najas flexilis* fitness. It is a perennial, has anchored submerged leaves, and flowers early in the growth season, it does not produce seeds in Britain and is not anemophilous, however it is epihydrophilous. If the hypothesis relating to the shading effect of flowering parts and their support structure is correct, epihydrophily would be just as important as anemophily and *Elodea* spp. would possess another key trait. However, if anemophily is important for production and dispersal of seed, *Elodea* spp. are lacking these traits as no seed is produced in Britain.

It is interesting that vegetative disseminule type is not significant, as it is the ability of *Elodea* spp. to reproduce vegetatively that has often been attributed to its success as an alien invasive. It is probable that as a dioecious alien invasive it is the ability to reproduce and hence colonise new sites without the need of a plant of the opposite sex, which is very important. However, this is not necessary in hermaphrodite plants or native populations where both sexes are present.

Unfortunately the ability to utilise bicarbonate and other more functional traits could not be included in this analysis due to a lack of information on the possession of these traits amongst the British aquatic flora, however inclusion of such traits would improve this technique's predictive ability particularly with respect to competition for nutrients and CO_2. This is very important, as eutrophication is one of the major threats to mesotrophic lakes, the systems where *Najas flexilis* grows. The lack of nutrient limitation could be expected to result in an increase in species which can respond to these nutrients by increasing their growth rate i.e. Grime's competitor species. An increase in the vegetative productivity of these lakes will also lead to greater competition for CO_2, with those capable of utilising other carbon sources most likely to outcompete the rest.

4.5 Conclusion

This approach does not explain the mechanisms by which different traits interact with *Najas flexilis* fitness. Experiments would be required to elucidate these mechanisms, now that at least some of the traits that do have a relationship with *Najas flexilis* fitness are known. This approach illustrates which traits are important within the environment that *Najas flexilis* grows. *Elodea* spp. do appear to possess many of the traits which do have a relationship with *Najas flexilis* fitness, but not all of them. Before deciding on the extent of the threat of *Elodea* to *Najas flexilis* it is important to understand further the importance of pollination mechanism and reproduction via seed in macrophyte communities.

Summary

- A generic trait based methodology can explain some of the variation in the growth of the target species.
- The quantity of wind pollinated, June-July flowering perennials in the *Najas flexilis* micro-habitat can explain 68% of the variation seen in *Najas flexilis* leaf area/shoot length. Possible reasons for this is that early (June –July) flowering plants and perennials

will have begun to grow before *Najas flexilis* germinates and will thus be able to effect *Najas flexilis* growth by shading and utilising resources before *Najas flexilis* grows. A possible reason for wind pollinated plants effecting *Najas flexilis* growth is that they must grow to the water surface to support their flowers above it, and this growth to the water surface will also shade *Najas flexilis.*

- *Elodea* spp. in Britain possess the perennial and June-July flowering traits, but are epihydrophilous.

5. Genetic variation in *Najas flexilis:* a hydrophilous annual in Scotland

5.1. Introduction

5.1.1 Aims

As Scotland is the European stronghold for *Najas flexilis*, it is important for conservation purposes to have some understanding of its population genetic structure. This can be addressed by asking two questions:

I. Is the genetic variation held mostly within or among populations?

II. Does the variation correlate with demographic or ecological factors?

This is particularly important if reintroduction of *Najas flexilis* is to be carried out, where the plant has become extinct, providing water quality can be improved.

5.1.2 Background

Najas flexilis occurs as both a diploid and a tetraploid with chromosome numbers of 2n = 12 or 2n = 24 respectively (Chase, 1947). Chase (1947) also noted that tetraploids were more robust than diploids. In Europe only the tetraploid has been found and this is also the most common form in Eastern North America. The diploid is found in both America and Canada (Love & Love, 1958). Both ploidy races appear to be fully fertile (Chase, 1947).

5.1.3 Factors influencing the pattern of genetic variability in *Najas flexilis*

Life history traits have been related to the partitioning of genetic variation within and among populations (Hamrick & Godt, 1996; Nybom & Bartish, 2000). Hamrick & Godt (1996) related life history traits to the results from isozyme analysis on 459 species and Nybom & Bartish (2000) related them to the results from 84 papers on RAPD analysis (including one hydrophile). The two studies found similar results (Table 5.1). Factors which appear to affect the partitioning of genetic diversity are; taxonomic status, life form, geographic range, breeding system and successional status (Hamrick & Godt, 1996; Nybom & Bartish, 2000). The main difference between the two data sources was that RAPD analysis showed significant differences depending on the geographic range of the species involved, whereas isozyme analysis did not.

Table 5.1 The affect of life history traits on the partitioning of diversity a) among, and b) within populations, as found from allozyme (A) studies summarised in Hamrick & Godt (1996) and from RAPD (R) studies summarised in Nybom & Bartish (2000). Nybom and Bartish looked at both ϕ_{st} and G_{st} to look at among population diversity, where these results differ it is noted in the table. Hamrick & Godt (1996) also used G_{st} to look at among population differences and used H_{es} to look at within population differences whilst Nybom and Bartish (2000) used H_{pop}.

a) Among populations

Characteristic	Method	Low level of genetic diversity	High level of genetic diversity
Taxonomic status	A	Gymnosperms	Angiosperms
	R	No significant differences	
Life form	A	Long lived perennials	Annuals
	R	Short & long lived perennials	Annual (only ϕ_{st} significant)
Geographical range	A	No significant differences	
	R	Endemic	Regional & widespread (only ϕ_{st} significant)
Regional distribution	A	Boreal-temperate	Temperate and tropical
	R	Not investigated	
Breeding system	A	Outcrossing wind pollinated	Selfing
	R	Mixed & outcrossing	Selfing
Seed dispersal	A	Gravity dispersed & animal attached	Gravity dispersed
	R	No significant differences	
Mode of reproduction	A	No significant differences	
	R	Not investigated	
Successional status	A	Late successional	Early & mid successional
	R	Late successional	Early successional
		Mid successional (G_{st})	Mid successional (ϕ_{st})

$G_{st}=(H_t-H_s)/H_t$ where H_s is the expected heterozygosity within all analysed populations and H_t is the expected hetrozygosity across the whole material.
ϕ_{st} is an estimate of between population variability using a multi locus approach.

b) Within Populations

Characteristic	Method	Low level of genetic diversity	High level of genetic diversity
Taxonomic status	A	Dicotyledons	Monocotyledons and Gymnosperms
	R	Monocotyledons & Dicotyledons	Gymnosperms
Life form	A	Short lived perennials and annuals	Long lived perennials
	R	Annuals	Short & long lived perennials
Geographic range	A	Endemic	Widespread
	R	No significant differences	
Regional distribution	A	Boreal-temperate	Temperate and tropical
	R	Not investigated	
Breeding system	A	Selfing, mixed-mating animal pollinated	Outcrossing species
	R	Selfing	Mixed & outcrossing
Seed dispersal	A	Explosively dispersed	Animal attached and wind dispersed
	R	No significant differences	
Mode of reproduction	A	No significant differences	
	R	Not investigated	
Successional status	A	Early successional	Late successional
	R	Early & Mid successional	Late successional

Aquatic plants, particularly hydrophiles, have often been grouped together in the past as plants that have a similar partitioning of genetic variability. Isozyme analysis of a number of hydrophiles show low levels of variation within a population compared to higher levels between populations (Barrett *et al.*, 1993; Hollingsworth *et al.*, 1996; Laushman, 1993; Les, 1988).

However, it should be noted that among hydrophilous plant species there are a broad range of life history traits. Hydrophilous species include monoecious and dioecious taxa, some of which are capable of clonal growth and some which are not, they include annuals and perennials, which occupy various successional stages and can be widespread or local taxa. Hamrick & Godt (1996) and Nybom & Bartish (2000) show that life form, geographic range, breeding system and successional status can all effect the partitioning of genetic diversity. Therefore, it appears more logical to consider the partitioning of genetic diversity in the context of all life history characteristics rather than just the medium in which pollination occurs.

Table 5.2 Expected patterns of population diversity according to the life history traits of *Najas flexilis*. The level of diversity is in comparison to the average values of other species sharing these traits (Nybom & Bartish, 2000).

Life history characteristic	*Najas flexilis* trait	Within population diversity	Between population diversity
Taxonomic status	monocotyledon	low	high
Life form	annual	not significant	high
Geographic range	not endemic	not significant	high
Breeding system	selfing	low	high
	mixed*	high?	low?
	outcrossing*	high?	low?
Seed dispersal	ingested	not significant	not significant
	water	not significant	not significant
Successional status	early	low	high

* Between population diversity is predicted to be low in outcrossing and mixed terrestrial flowering taxa due to interpopulation pollination, however hydrophily does not allow easy transfer of pollen between populations if they exist in discrete water bodies.

Table 5.2 shows the expected partitioning of genetic diversity in a species with the biological attributes of *Najas flexilis* according to the RAPD literature survey by Nybom & Bartish (2000). If the predicted levels of diversity from Nybom & Bartish (2000) are correct it can be expected that *Najas flexilis* will hold most of its genetic diversity between populations rather than within them, although whether or not *Najas flexilis* has a predominantly selfing or mixed breeding system is obviously important.

5.1.3.1 Breeding system

Hutchinson (1975) noted that the majority of reproduction amongst aquatics was asexual, with the exception of a small group of annuals such as *Najas.* Such a tendency for clonal growth amongst aquatics could limit genetic variability within a population. However, the majority of the Najadaceae are annuals, (Les, 1988) and do not exhibit clonal growth, the only exception is *Najas marina,* which has been reported to produce turions (Agami *et al.*, 1986).

Reproduction in *Najas flexilis* is thus obligately sexual, with seed produced from monoecious flowers. Dicliny could have evolved to avoid selfing and inbreeding depression, with monoecy potentially representing an intermediate stage between hermaphrodite and dioecious plants. However, monoecy does not necessarily prevent self-pollination via geitonogamy unless it is coupled with temporal or spatial separation of male and female flowers. An example of a predominantly selfing monoecious hyphydrophile is *Zannichellia* which has a floral biology which makes xenogamy seem unlikely (Les, 1988). In *Zannichellia,* the anther arches over the funnel shaped stigma and drops the pollen directly onto it in a gelatinous mass (Haynes & Holm-Nielsen, 1987). In contrast, another monoecious hydrophile *Zostera marina* has been found to have high outcrossing rates (mean t = 0.862 across 4 populations). However, outcrossing rates dropped when the amount of clonal growth increased, as pollen could not reach non-clone mates (Reusch, 2001 and references therein).

An alternative to monoecy evolving from hermaphroditic flowers to promote outcrossing, is that dioecy could be the ancestral state in *Najas* and that monoecy has evolved in *Najas flexilis*, perhaps under selection for reproductive assurance. In clonal dioecious species if sex ratios are skewed, or in extreme cases one sex is absent, vegetative propagation allows for perpetuation and dispersal. An example is unisexual populations of *Elodea* species in Britain, where sexual reproduction is impossible, but vegetative propagation ensures effective perpetuation and dispersal (Simpson, 1988). In contrast in an annual dioecious species, if sex ratios are highly skewed, mate availability may become limiting which can lead to a drastically reduced effective population size. For instance, the dioecious species *Najas marina* can show asymmetry in the number of males and females in Britain and turions have not been found in these populations (Handley & Davy, 2000). In contrast to *Najas marina* in Britain the monoecious state of *Najas flexilis* should ensure a ready supply

of males and females. However, information on resource allocation and spatial and temporal positioning of flowers is lacking. Evidence that self-pollination can occur comes from the observation that isolated plants set seed in cultivation. However, the extent to which this occurs in the wild is unknown.

There have been no specific studies on the pollination of *Najas flexilis,* although there have been reports of temporal and spatial separation of male and female flowers, which if correct might be expected to favour outcrossing. Preston & Croft (1997) state that the male flowers are to be found in the upper nodes of the plant with the female flowers below. Triest (1988) states that *Najas flexilis* have male and female flowers predominantly in different nodes with the males positioned more to the top. However, occasionally the male and female flowers can be in the same nodes on the top of the plant. However, Polsluszny and Sattler (1976) found male and female flowers with no preference for positioning on the plant. They also stated that only one flower was to be found in each leaf axil. However, Stace (1997) states that one to three flowers could be found in a single node. Such conflicting reports makes drawing any conclusions impossible without further studies.

The quantity and form of the pollen will also affect chances of pollination. Triest (1988) found that in comparison to the 4-locular dioecious *Najas* species, the monoecious *Najas* species either had smaller 4-locular anthers or were 1-locular. He suggested this was because less pollen is required when flowers are in close proximity. It would be interesting to compare pollen:ovule ratios of *Najas flexilis* with dioecious species such as *Najas marina* to test this hypothesis.

The pollen in *Najas* species has been reported to begin to germinate on liberation, the elongate shape of the pollen tube increases the surface area and thus increases the chance of it coming into contact with the long forked stigmas (Hutchinson, 1975; Triest, 1988). *Najas flexilis* is a prolific seed producer with a seed found in almost every node of the plant; an average of all plants studied showed that there is approximately one seed every 1.25cm of plant length. This indicates that reproduction is not generally pollen limited. This level of reproductive success shows that regardless of whether *Najas flexilis* is selfing or xenogamous the pollination mechanism can be successful.

5.1.3.2 Levels of between population gene flow

Dispersal between habitats is intrinsically difficult when life is restricted to ponds and lakes, due to the concept of lakes being aquatic islands in a terrestrial sea, and *Najas flexilis* is no exception. The dispersal unit for *Najas flexilis* are its seeds. External attachment dispersal (e.g. fishermen, birds feet) or wind dispersal are unlikely, because the seeds are intolerant to desiccation (Hay & Muir, 2000), this trait is not shared with all aquatics. *Najas flexilis* seeds are reported to be eaten by wild fowl (Martin *et al.*, 1961) and widgeon (Knapton & Pauls, 1994), these accounts are both from the North American continent. Following ingestion by a bird, seeds have to pass through the digestive system in a viable state and be deposited in a suitable habitat. If the seeds are dispersed a long distance they may lack the necessary local adaptations to become established. Instead, more localised movement through interconnected water bodies by streams and rivers could be an efficient means of dispersal.

5.1.3.3 History and ecology of *Najas flexilis* in Britain

The plants' history in Britain is also an important factor to consider regarding genetic variation in *Najas flexilis*. *Najas flexilis* has a circumboreal range, however the majority of its populations occur in Northern America. The sparse disjunct European range of *Najas flexilis* is thought to be a recent Flandrian product, possibly due to a decrease in temperature since the plant was at its most abundant at the time of the thermal maximum in zone VI of the late Flandrian. With the exception of sites in Perthshire, it is now restricted in the British Isles to the Atlantic fringe (Godwin, 1975).

The other reason suggested by Godwin (1975) for the restriction of *Najas flexilis* distribution since boreal times is the lack of appropriate habitats. In particular, the number of calcareous or basic lake basins unaffected by drainage and other human interference, such as eutrophication has been reduced. This has also been documented by fieldwork at former *Najas flexilis* sites in Perthshire, which have suffered from eutrophication (see Chapter 2). The occurrence of eutrophication could represent bottlenecks for the surviving populations. The annual nature of the plant makes it particularly sensitive to environmental change, and year to year fluctuations in number could promote genetic drift and loss of variability. Genetic depauperacy at the edge of species ranges in geographically peripheral populations is a common phenomenon (Levin, 2000). Water depth might also influence patterns of genetic variability in *Najas flexilis*. It is in the shallow depths where xenogamy is most likely

to occur for two reasons. The first is the greater turbulence experienced by the plant in shallow waters, thus increasing pollen and seed movement, increasing the chance of mating with non-self or non-sibling plants, the second is the increased density of pollen (Les, 1988), generally increasing the chances of pollination. This would lead to a possible scenario, where plants would most likely be selfing or mating with siblings in the still depths and sheltered areas of the lake, and outcrossing and mating with less related plants in the more turbulent shallows and exposed parts of the lake. In America the plant can be found at depths as diverse as 0.2m-14m (Sheldon & Boylen, 1975). It is noteworthy that in Britain *Najas flexilis* is rarely found in the immediate littoral zone and instead occurs in sheltered deeper water usually greater than 1.5 m (Preston & Croft, 1997). If the speculation about deeper water and shelter leading to increased selfing is correct, this scenario of selfing might be typical of the sheltered waters in which *Najas flexilis* grows in the British sites.

5.1.3.4 Summary

A general prediction that has emerged considering the biological features, history, ecology, and current distribution of *Najas flexilis* in the UK is low within, and high between, population diversity. To assess this molecular markers have been employed to examine partitioning of genetic variation in *Najas flexilis* in Britain.

5.2 Methods

5.2.1 DNA collection and extraction

The original intention was to collect 5 plants from a range of Scottish and Irish sites and 20 plants from a subset of these sites, at least 5. With this aim samples were collected from the sites surveyed in 1999 (Table 3.1). After collection, the leaves were removed from the plants, divided into two, and dried with silica gel. This was to provide a back up sample if anomalies occurred.

DNA was then extracted using a modified method from Doyle & Doyle (1990). 350 µl of 2 X Cetyl Trimethyl Ammonium Bromide (CTAB) buffer with 0.2% β-mercapto-ethanol preheated to 65°C was added to each sample along with a pinch of PVPP (Polyvinylpolypyrrolidone) and a pinch of acid washed sand. The sample was then homogenised, and a further 350 µl of 2 X CTAB buffer with 0.2% β-mercaptoethanol was then added. The sample was mixed and then incubated for 1 hour at 65°C. The samples

were cooled to ambient temperature and 700 µl 24:1 chloroform:isoamylalcohol was added. The samples were mixed for 30 minutes, and then centrifuged for 20 minutes at 13 000 rpm. The supernatant was then collected and the process was repeated. The supernatant was removed for a second time, and the DNA precipitated by adding 400 µl of freezer cold isopropanol, and the samples were mixed gently and then left overnight in the -20°C freezer. The samples were then centrifuged at 13 000 rpm for 15 minutes to allow a DNA pellet to form. The isopropanol was then removed, 200 µl of 70% ethanol was added to wash the pellet and the pellet was dislodged from the eppendorf. This was spun down for 5 minutes at 13 000 rpm, and the ethanol was removed and the cleaned pellets were allowed to air-dry. The DNA was then dissolved in 100 µl of distilled water.

Although DNA that could be visualised on a 1% agarose gel could be obtained from the above DNA extraction protocol, it would not necessarily PCR with RAPD, Inter SSR, or chloroplast primers. In contrast, the internal transcribed spacers (ITS) of ribosomal DNA markers were generally successfully amplified from plants collected at the start of the *Najas flexilis* growth season, but their success deteriorated as older plants were used. Occasional individual DNA samples amplified for all the markers tried, although the number of such samples was minimal despite the same extraction method being used on all specimens. The use of QUAIGEN DNAeasy kits or additional sodium acetate washes and reprecipitation did not aid the success of PCR.

Therefore, new samples were collected (July-September 2000). For each sample, a single plant was collected, and the reproductive structures and roots removed. The plant was then blotted dry with absorbent paper and dried in mostly non-indicating silica gel, with a small amount of indicating silica gel. Samples were stored in plastic bags with an airtight zip seal at room temperature.

These new samples were snap frozen before grinding, this appeared to greatly increase grinding capacity and therefore the quantity of DNA extracted. The new samples exhibited the same problems as their predecessors. The occasional DNA samples, which were successfully replicated by PCR, when mixed with equal quantities of DNA samples that did not successfully PCR, produced intermediate results. This suggested inhibitors in the samples, again the above cleaning methods were attempted without success. However diluting the samples 10 fold appeared to decrease the concentration of inhibiting substances

sufficiently without decreasing the DNA concentration detrimentally, this resulted in successful PCR with RAPD primers. Not all samples replicated successfully under this treatment particularly the samples from older plants. This suggests that PCR inhibiting substances may increase in the plant with age.

Only sixty three samples were successfully amplified from 13 sites covering the geographic range of *Najas flexilis* within Britain and Ireland; this represents 5 samples from all but two sites where only four samples could be amplified. Five samples were also amplified from one site in Lake George, New York State for comparative purposes (Table 5.3). This is a big reduction from the original sampling strategy, especially with regard to the number of samples from a site, reducing the ability to answer the question of within population diversity.

Table 5.3 Sites from which *Najas flexilis* was collected

Sites	Abbreviation	Grid reference
Mainland		
Lake of Menteith	lom	27577002
Loch Clunie	cl	37116444
Loch of Craiglush	cr	37044446
Outer Isles		
Loch Druidibeg	dr	08780388
Loch nan Capull	nc	08754161
Loch with no name	lo	08766369
Loch na Cuithe Moire	cm	08738234
Inner Isles and Kintyre		
Loch Gorm	go	16230655
Loch Lossitt	los	16408652
Loch Nan Gad	ng	16783573
Ireland		
Lough Leane	le	14768203
Cregduff Lough	cd	02718392
Mullaghderg Lough	md	00932890
USA		
Lake George	us	NY state

5.2.2 RAPDs

A subset of eight samples representing the geographical range of sites was used to screen RAPD primers. The RAPD primers used were 10-base primer kits A, F and P from Operon technologies. From this screening 9 primers (Table 5.4) were chosen for their ability to produce clear reproducible bands in this sample set.

Table 5.4 Primers selected for use.

Primer code	Sequence
OPA19	5'-CAAACGTCGG-3'
OPP5	5'-CCCCGGTAAC-3'
OPF3	5'-CCTGATCACC-3'
OPP16	5'-CCAAGCTGCC-3'
OPP8	5'-ACATCGCCCA-3'
OPF14	5'-TGCTGCAGGT-3'
OPA9	5'-GGGTAACGCC-3'
OPA7	5'-GAAACGGGTG-3'
OPP17	5'-TGACCCGCCT-3'

Each sample was diluted 10 fold before PCR. The reaction mixture per sample had a total volume of 25 µl and contained: 2.5 µl ammonium sulphate buffer (160mM); 2.5 µl dNTPs (2mM); 1.25 µl magnesium chloride (50mM); 2.5 µl primer (5µM); 1.75 µl DNA template; 0.2 µl Taq polymerase; 13.44 µl water; 0.5 µl formamide (100%); 0.36 µl bovine serum albumin (BSA). Negative and positive controls were included in every PCR, four samples were both replicated within every PCR and run on every gel to aid scoring. The thermo cycle was: 2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min at 37°C, 2 min at 72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, 2 min at 72°C; 41 cycles of 30s at 94°C, 1 min at 35°C, 2 min at 72°C followed by a final 5 min extension at 72°C. The products were run on a 1.6 % agarose gel containing ethidium bromide in 1 X Tris-borate EDTA. A 1kb ladder was used as well as the replicate samples to aid scoring. Bands were scored for presence/absence.

5.2.3 Data analysis

The presence/absence data matrix generated using the 9 RAPD primers was converted to a similarity matrix using Jaccards similarity index: $SJ = c/(a + b + c)$, where c is the number of bands shared by the two samples and a and b are the numbers of bands unique to each of the samples (Jaccard, 1908). In calculating this measure of similarity between pairs of samples, only presence of RAPD bands is taken into account. The absence of a RAPD fragment may be caused by a number of factors, thus using shared absences to represent similarity may misrepresent relationships (Coleman *et al.*, 2000; Weising *et al.*, 1995). The relationships of inter-individual similarity in multidimensional space were examined by principal co-ordinates (PCO) analysis on the Jaccard's similarity matrix using the 'R' package, provided by Phillippe Casgrain and Pierre Legendre. Neighbour joining trees, based on Nei and Li's (1979) distance, (again not using shared absence to indicate similarity) were produced in

PAUP (Swofford, 2001). To assess levels of intra-population variation, the proportion of bands that were polymorphic was calculated.

Preliminarily, Detrended Correspondence Analysis (DCA) (Hill, 1980) was carried out on the RAPD data set. This yielded axis lengths greater than 4 S.D. so it was decided to proceed with a Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). CCA was used to investigate the relationships between the environment and the RAPD data and between the morphometric (Trait data) and RAPD data. The morphometric data used was the trait data (see section 2.2.2.1), and the environmental data used was the water and sediment chemistry data (see section 2.2.3). As the environmental data and morphometric data were not directly related to an individual, but to the lake population as a whole, treating the RAPD data as phenotypic characters it was converted to the frequency of band occurrence within a population. This is an oversimplification, as the band frequencies on the gel do not reflect the allele frequency in the population. However, determination of actual allele frequencies in the population, with no data on the breeding behaviour, such as a F_{IS} value, coupled with the tetraploid state and no evidence whether it is an auto or allopolyploid is extremely difficult. This band frequency data was used in the same way as community composition data is normally used in CCA, i.e. this is the primary data set. The environmental and morphometric data were used as the external variables (the secondary data set), which constrains the RAPD data in CCA. These are normally habitat factors but can equally be phenotypic characters (Prentice & Cramer, 1990). A Monte Carlo permutation test was then used to test the extent to which variation in the RAPD data was significantly related to variation in the external variables, in this case environmental variables or morphometric variables.

In order to investigate whether the variability of a population is related to its position in a loch, the percentage of polymorphic loci per population was regressed with exposure. Exposure was calculated as in section 2.2.3. This was then divided by log of the depth of the plants plus one, to take into account that with depth the exposure experienced by the plant will decrease.

The results from this regression will again be limited by the lack of in depth meteorological measurements of wind speed at each site (see section 2.2.3). An improvement in the measurement of exposure would make the analysis more accurate. An increase in the

number of samples per site would also increase the accuracy of the assessment of genetic polymorphism.

5.3 Results

5.3.1 Genetic diversity

A total of 63 reproducible and polymorphic bands were resolved, this is reduced to 49 if the American samples are excluded. All but two individuals, from loch with no name in S. Uist, could be distinguished as an individual genotype. The number of polymorphisms within the populations is shown in Table 5.5. Two separate neighbour joining analyses were carried out, one including and one excluding the American site. The greatest genetic distance in the neighbour joining tree for all sites was that between the American and the Scottish and Irish sites. Little other separation of samples can be elucidated from Fig. 5.1, because the other sites are all clustered in comparison to the large genetic distance between the sites on either side of the Atlantic.

Table 5.5 Intra population RAPD polymorphism

Sites	Number of polymorphic bands	% of polymorphic bands present in Scotland and Ireland	% of polymorphic bands present in Scotland, Ireland and USA
Mainland			
Lake of Menteith	9	18.4	14.3
Loch Clunie	11	22.4	17.5
Loch of Craiglush	14	28.6	22.2
Outer Isles			
Loch Druidibeg	7	14.3	11.1
Loch nan Capull	4	8.2	6.3
Loch with no name	4	8.2	6.3
Loch na Cuithe Moire	13	26.5	20.6
Inner Isles and Kintyre			
Loch Gorm	5	10.2	7.9
Loch Lossitt	6	12.2	9.5
Loch Nan Gad	5	10.2	7.9
Ireland			
Lough Leane	7	14.3	11.1
Cregduff Lough	8	16.3	12.7
Mullaghderg Lough	4	8.2	6.3
USA			
Lake George	13		20.6

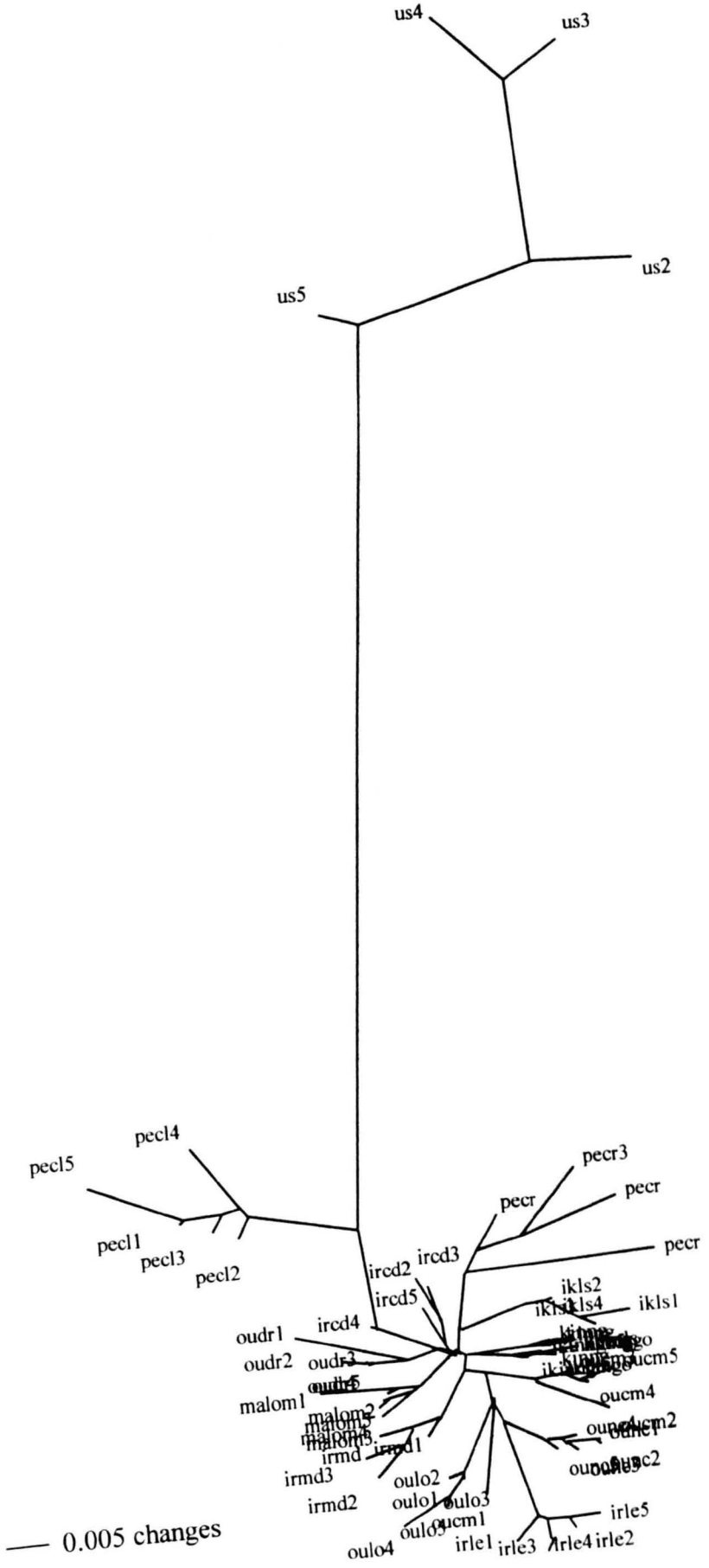


Figure 5.1 Neighbour joining tree showing the relationship between the RAPD genotypes for samples from USA, Britain and Ireland. Abbreviations are for sites are in Appendix 1 with the additional prefixes of ma = mainland, ik = Inner Isles and Kintyre, ou = Outer Isles and ir = Ireland

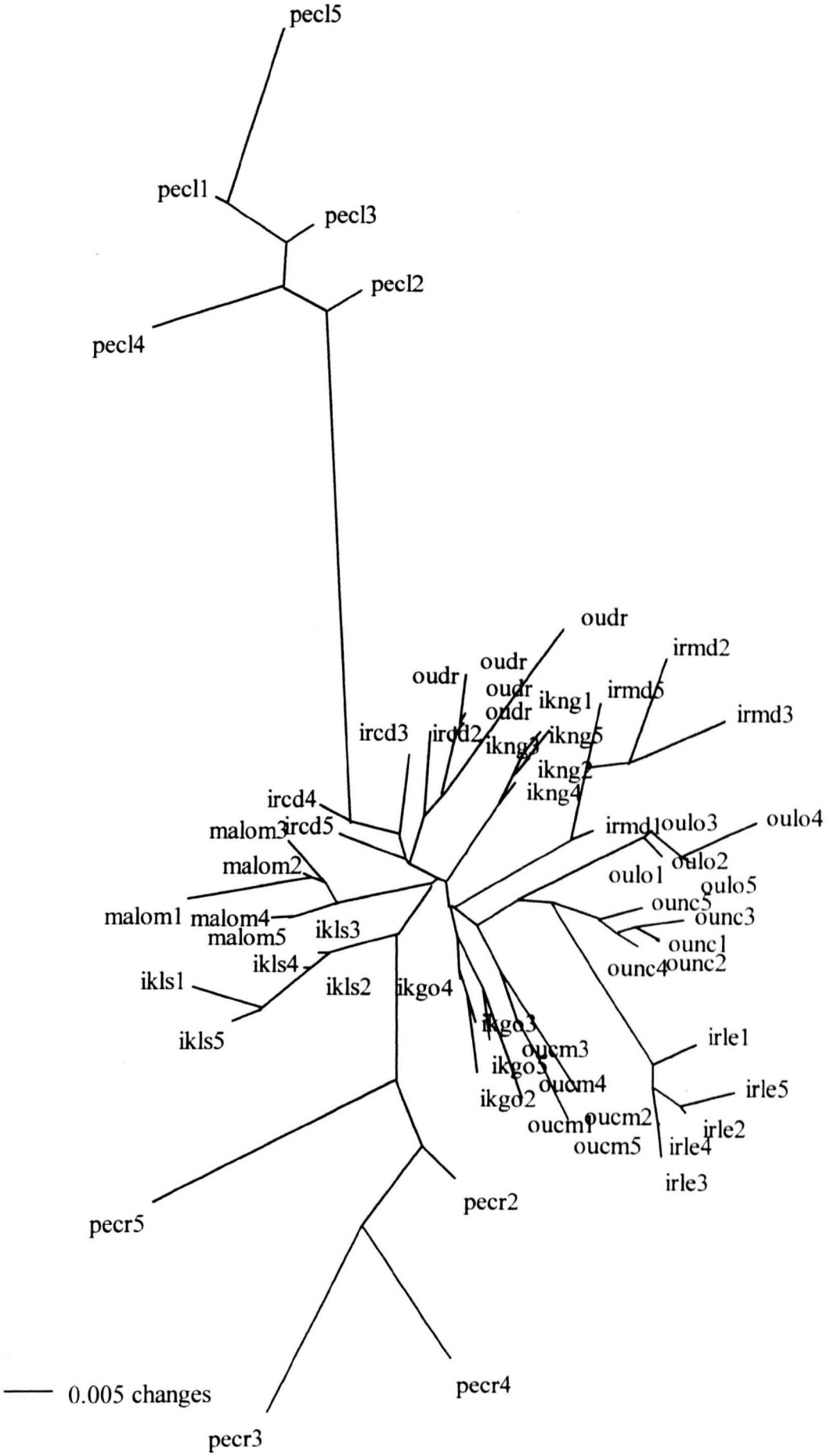
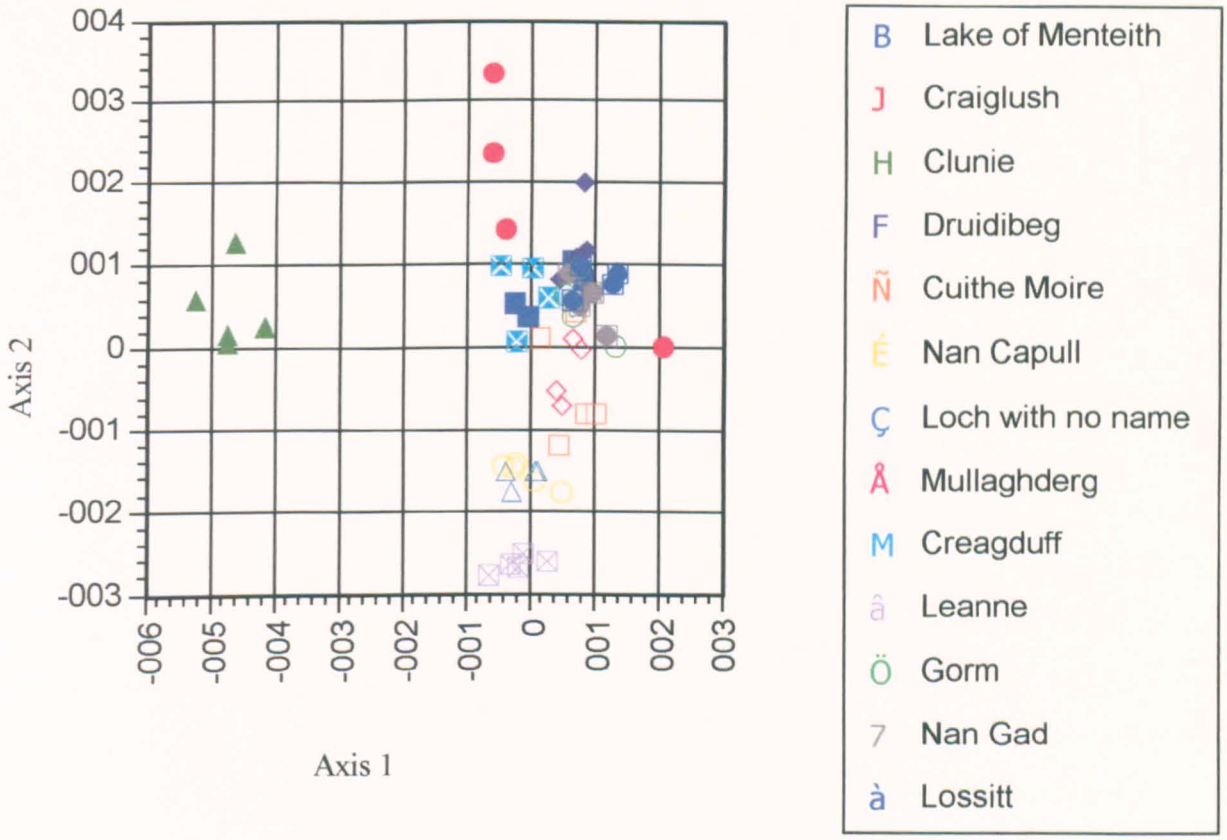


Figure 5.2 Neighbour joining tree showing the relationship between the RAPD genotypes for samples from Britain and Ireland Abbreviations are for sites are in Appendix 1 with the additional prefixes of ma = mainland, ik = Inner Isles and Kintyre, ou = Outer Isles and ir = Ireland

For the analysis excluding the American samples both the PCO (Fig. 5.3) and neighbour joining tree (Fig. 5.2) show similar results. The greatest genetic distance is between the two sites in Perthshire, particularly Loch Clunie and the remaining sites. It is also apparent that partitioning of genetic variation is mostly between populations rather than within them. This is illustrated clearly by the neighbour joining tree (Fig. 5.2), where all samples within a site are connected to the same branch. However, the genetic distance between these populations is not large. The PCO analysis (Fig 5.3) shows that although samples are most similar to samples from the same population the populations are not greatly different from one another.



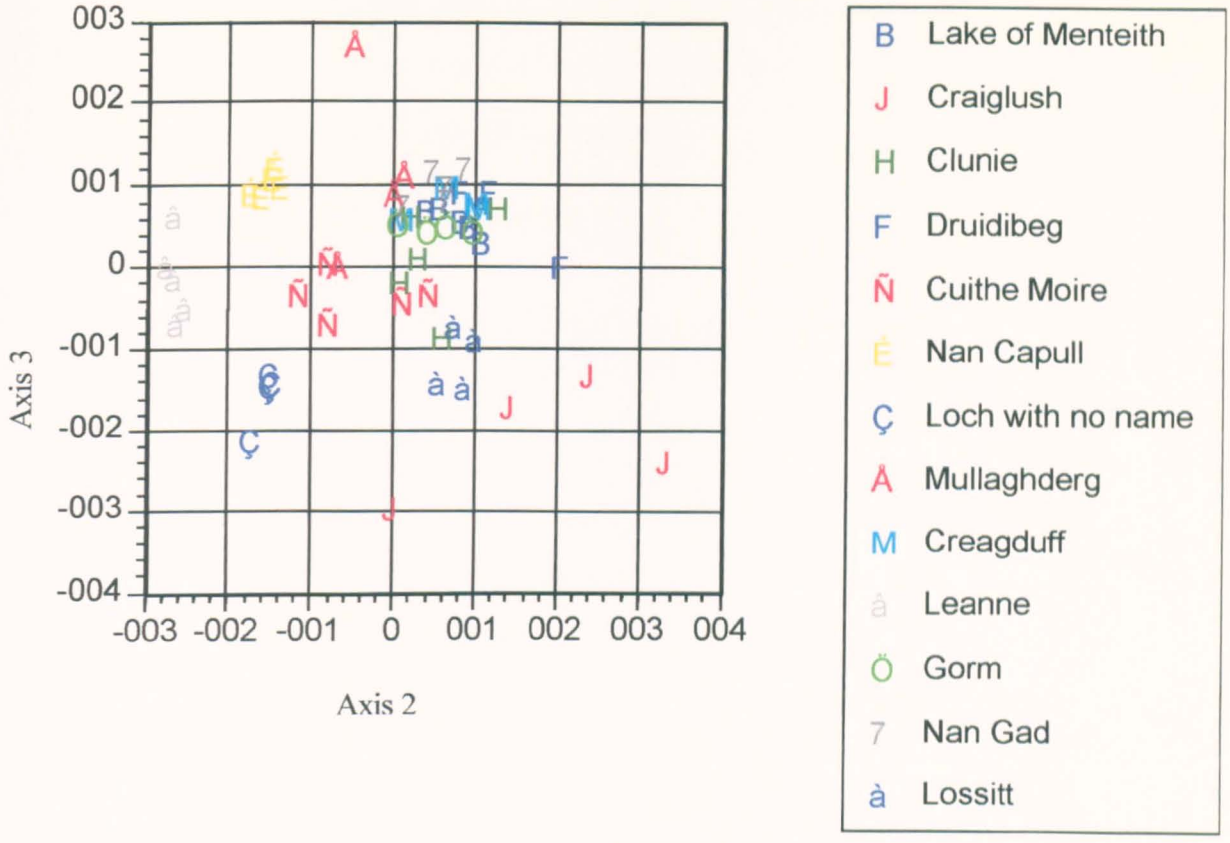


Figure 5.3 PCO plot showing the relationship between the RAPD genotypes for samples from Britain and Ireland.

5.3.2 Relationships between the genetic properties of a population and the environment and morphological traits of the population

The Monte Carlo permutation tests showed that the relationship between the environment or morphometric variables and the gradients of change in the RAPD data extracted by CCA was not significant (Table 5.7 & 5.9). Therefore, there is no reason to reject the null hypothesis; that there is no correlation between variation in RAPD data and the environment or morphometric measurements. Although 43.9% of RAPD-environment relationship is explained in the first two axis of this CCA analysis, that only explains 28.4 % of the total variation in the RAPD data (Table 5.6). The relationship between the morphometric measurements and the RAPD data is similar, with 45.4 % of the RAPD-morphometric relationship explained in the first two axis, but only 29.6 % of the total variation in the RAPD data explained (Table 5.8).

Table 5.6 Cumulative variance for the first four axes of CCA ordination

	Axes			
Cumulative percentage variance:	1	2	3	4
of RAPD data	15.9	28.4	37.1	45.6
of RAPD-environment relation	24.7	43.9	57.4	70.5

Table 5.7 Results of the Monte Carlo permutation tests for RAPD-environment relationships

Variable	P value	F statistic
pH	0.420	1.07
N-TON	0.340	1.17
K	0.445	1.01
P-TP	0.465	0.97
Conductivity	0.560	0.92
Light	0.380	1.10
P-DRP (PO_4)	0.570	0.85
N-NH_3	0.580	0.72

Table 5.8 Cumulative variance for the first four axes of CCA ordination

	Axes			
Cumulative percentage variance:	1	2	3	4
of RAPD data	15.6	29.6	39.0	46.8
of RAPD-morphometric relation	24.0	45.4	59.8	71.7

Table 5.9 Results of the Monte Carlo permutation tests for RAPD-morphometric measurement relationships

Variable	P value	F statistic
Number of reproductive structures/shoot length	0.270	1.13
Leaf area/shoot length	0.545	0.93
Number of reproductive structures/number of branches	0.150	1.31
Shoot weight/shoot length	0.270	1.24
Root area/root length	0.395	1.07
Weight of Reproductive Structures/number of reproductive structures	0.450	1.01
Number of branches/shoot length	0.525	0.87
Weight of reproductive structures/shoot length	0.740	0.57

5.3.3 The relationship between the genetic variability of a population and its environment

The regression of exposure with number of polymorphism per population shows there is a direct relationship between the two (Fig 5.4). With increased exposure, an increase in the number of polymorphic bands is seen. However there is still a relatively large amount of noise in the data presented, this is probably due in part to the exposure calculation method used and the small sample sizes.

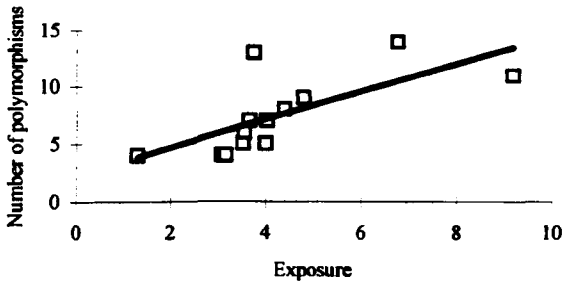


Figure 5.4 The relationship between number of polymorphisms per population and exposure. $r^2 = 0.476$ $p < 0.01$ where Number of polymorphisms =1.2Exposure + 2.3

5.4 Discussion

Technical difficulties experienced working on the population genetics of *Najas flexilis* have resulted in a small samples size available for the final analysis. This naturally limits the power of inferences that can be drawn from the results, and hence all conclusions are tentative.

5.4.1 Partitioning between lakes

The fact that samples within a lake are most closely related to each other, rather than those from other populations, implies that geographic isolation is having an effect. This is in common with many hydrophilous plants (Barrett *et al.*, 1993; Les *et al.*, 1997). It is however quite surprising that the populations in South Uist are not more similar to each other. This island contains at least 20 extant *Najas flexilis* lochs all of which are in close proximity in a habitat mosaic with many potential dispersal corridors. The obvious separation of Loch Clunie and Craiglush in Perthshire is also surprising. These two lochs are part of a chain of five lochs interconnected by the Lunan Burn. All five lochs in the chain have been reported in the past to contain *Najas flexilis,* however the last loch in the chain

appears to be too eutrophic to support a *Najas flexilis* population now. Craiglush is the first loch in the chain and Clunie the fourth, a distance of less than 9 km. This obvious difference between the two lochs implies that a single loch is the population unit for this species in the geographical area studied. There does not appear to be any relationship between geographical distance and genetic distance. It is then likely that each population has become distinctive due to genetic drift. This would explain the overall low genetic distance between populations, but the distinct population units.

5.4.1.2 Genetic variability within populations

While it is not possible to make any strong inferences about reproductive biology from a small sample of individuals and a dominant marker data set, it is interesting to note that each individual of *Najas flexilis* except for one pair of samples could be identified as a separate genotype by the RAPD primers used. This implies that populations do not consist of completely homozygous and uniform selfing lines. This could be attributable to xenogamy, although without information on mutation rates of the variable loci and long term estimates of population sizes and migration rates, selfing (or partial selfing) cannot be excluded. Further studies should use co-dominant markers such as microsatellites to investigate average individual levels of heterozygosity, and also to undertake progeny genotyping (c.f. Reusch, 2000) to directly calculate outcrossing rates.

5.4.2 Relationships between genotype and the environment and morphological traits

The lack of a significant relationship between the environment and the RAPD data does not imply that there is no relationship between the genotype and the environment. RAPDs are neutral markers, which unless they are closely linked to an area of the genome that has an adaptation to the environment, will not show a significant relationship. The same is true for the lack of relationship between the RAPD data and the morphometric data. It was, however, worth investigating as neutral markers have been found to have a relationship with other plant traits. An example is that 5'-anchored simple sequence repeats have been related to a plant's germination traits (Marshall *et al.*, 2000). However, the results here show no relationship between the RAPD markers and plant performance traits or the environment.

5.4.3 Relationships between population variability and the environment

The fact that the number of polymorphisms per population is related to the exposure experienced by the plants is interesting. The greater exposure the plant experiences the more likely it is to break up in the autumn gales and spread its seed amongst the population. *Najas flexilis* plants do not float, so unless water movements pick them up and carry them around the loch the plant and its seed will sink where it grew and there will be no dispersal even within the loch. Lochs with a relatively large exposure often have *Najas flexilis* plants found in the drift in the Autumn, whereas those with low exposure often do not (personal observation from fieldwork 1998-2000). Other factors as well as exposure also will affect this, such as substrate type (allowing plants to be uprooted) and the topography of the lakebed (plants rarely appear to reach the drift line if the lake bed suddenly shelves).

Pollen movement is also going to be affected; without exposure or waves, the pollen will sink and geitonogamous selfing will be the most likely result. However, if the water is moving the pollen has a greater chance of finding a non-self or sibling stigma. The formula used for calculating exposure contains a depth function, so those plants occurring in shallow depths have a greater exposure measure. Plants occurring in shallow water are not only benefited by the greater exposure measure, but also because pollen will be denser than in deep water and therefore the chances of pollination will increase. A simple test of this last hypothesis would be to compare the proportion of flowers that set fruit in marginal versus deep water zones.

5.5 Conclusions

The primary step in any reintroduction programmes for *Najas flexilis* is environmental restoration. This is a fundamental prerequisite to translocation; otherwise, the introduced individuals are likely to suffer the same fate as their extirpated predecessors. When the environmental conditions are suitable, it is then necessary to consider which populations to use for donor propagules. Populations that are geographically proximal and ecologically similar would the primary candidates. Donor populations should be of a large size to be able to withstand seed removal. Sampling large populations should also decrease the likelihood of reintroducing plants suffering from inbreeding depression. Selection is less efficient in small populations in weeding out less-fit individuals.

In terms of using the molecular data generated during this study to identify donor populations, inferences are hampered by the small final sample sizes and the lack of clear geographical structure detected. At present, the most appropriate criteria for reintroduction programmes should be based on ecological data.

Summary

- RAPD primers were used to look at the genetic diversity of *Najas flexilis*, specifically in Scotland and Ireland.
- All but two individuals could be identified as different genotypes.
- Individuals were generally more similar to other individuals within the same lake than individuals outwith the lake, suggesting that the lake populations are relatively isolated with little movement of pollen or seeds between them.
- No obvious geographic structure could be detected in the *Najas flexilis* populations.
- No significant relationship was found between the water and sediment chemistry of the lake or the *Najas flexilis* trait data and the frequency of RAPD bands within a population.
- A direct relationship was found between the number of polymorphisms within a population and the exposure experienced by that population. This is most likely to be due to an increase in the movement of seeds and pollen due to an increase in water movement. This would result in a greater amount of cross pollination instead of self pollination and a greater chance of outcrossing with a non sibling plant.

6.The germination ecology of *Najas flexilis*

6.1. Introduction

6.1.1 Aims

To determine the environmental factors controlling *Najas flexilis* germination using laboratory based experiments.

6.1.2 Background

As an annual *Najas flexilis* is dependent for population survival on seed production, and the consequent successful germination of these seeds. The ripe fruits of *Najas flexilis* are shiny and dark brown-black in colour. The embryo makes up most of the fruit with a very thin translucent testa and a thin brittle epicarp (Hay & Muir, 2000). The shape of the seeds is described as elliptical, oblong (Triest, 1988). Surveys of plants across Scotland and Ireland have found that seed production varies, with 0-43 seeds found on a single individual. It has been found that seed number is dependent on a number of environmental factors including pH (Titus & Hoover, 1991; Titus & Hoover, 1993; and section 2.3.2.1).

Little has hitherto been known about the conditions required for germination. The seeds are produced from late summer to early autumn (August-October) and the seedlings begin to appear in early summer (June) in Scotland (personal observation). This phenology suggests a sensitivity of seeds to climatic conditions, which prevents immediate germination in autumn and stimulates germination in the summer. However, whether the seeds form a persistent seedbank beyond this short time period is unknown. Some of the possible mechanisms that may prevent or enhance germination are considered below with respect to aquatic conditions and species closely related to *Najas flexilis*.

6.1.2.1 Stratification

Muenscher (1936) found that *Najas flexilis* seeds had a stratification requirement. No germination was obtained from seeds stored in water at room temperature. However, seeds that had been stored in chilled water for one month germinated gradually over the following six months. Seeds that had been stored in chilled water for the past 7 months germinated uniformly and quickly (87% in six days). Wetzel & McGregor (1968) also found that germination of *Najas flexilis* was easily induced after a dormancy period of 15-30 days at

4°C. A stratification requirement for germination in *Najas flexilis* would delay germination from autumn, immediately after seed production, until the following summer.

6.1.2.2 Scarification

Germination in *Najas marina* has been found to improve with scarification (Agami & Waisel, 1984; Bone & Probert, 1994), which is often the product of ingestion, by animals. Agami & Waisel (1986) found that the ingestion of *Najas marina* seeds by mallard ducks improved germination to the same extent as mechanically cracking these seeds. Such physical dormancy, which is only broken by animal ingestion, is suggested to improve the dispersal of seeds. This is because seed ingested by a motile organism is often deposited away from where the parent plant grew. Dispersal can be a problem for aquatic species (see section 5.1.3.2) due to lakes being aquatic islands in a terrestrial sea, and such physical seed dormancy could help circumvent this problem. However this is a high risk strategy as ~70% of the ingested *Najas marina* seeds were digested by the mallards. Whether or not ingestion of *Najas flexilis* seeds by birds aids germination is unstudied. However, a study on lake Ontario showed a preference for *Najas flexilis* by foraging widgeons, which ate all parts of the plant (Knapton and Pauls, 1994). The preference of waterfowl for *Najas flexilis* was also noted by Martin *et al.* (1961).

6.1.2.3 Sediment conditions

The sediment and water chemistry can also be important for germination. Titus and Hoover (1991) found *Najas flexilis* seedlings emerged in greater abundance in alkaline silty lake sediment than in circumneutral organic pond sediment or acidic pond sediment. It was suggested that this could be due to organic growth inhibitors. Wetzel and McGregor (1968) embedded seed in agar, to obtain a suitable redox gradient and found that it did not appear to enhance germination in *Najas flexilis.* In contrast, Forsberg (1965) found that this did enhance the germination of *Najas marina.* Deoxygenated conditions have also been found to enhance the germination of other aquatic species such as *Zostera marina* (Moore *et al.*, 1993; Probert & Brenchley, 1999).

6.1.2.4 Light/dark

Burial is a hazard for seeds in both terrestrial and aquatic systems. It is important that seeds do not germinate if they do not have the resources to reach the substrate surface after burial

as then the seed is wasted. A requirement of light as a stimulus for growth prevents seed waste in such situations. An example of this is in *Myriophyllum spicatum* where seed germination is much reduced by burial (Hartleb *et al.*, 1993). *Zostera marina* germination is also enhanced by light (Probert & Brenchley, 1999). However, Wetzel & McGregor, (1968) found that light of 600 lux was distinctly inhibitory by an order of magnitude to germination of *Najas flexilis. Najas marina* has also been found to exhibit maximum germination levels in the dark (Bone & Probert, 1994). As *Najas flexilis* is often found in deep waters and in lakes with low water clarity, it is not surprising that it can germinate in situations with low light levels, or even in dark conditions. However, as *Najas flexilis* grows in flocculent sediment (section 2.1.4), the absence of a light requirement for germination will make burial and consequent seed wastage a hazard.

6.1.2.5 Temperature

Wetzel & McGregor (1968) found that the percentage germination of *Najas flexilis* seeds could be nearly doubled by exposure of surface sterilised seeds to heat (35 or 42°C) for 24 or 72 hours in the dark. Temperatures of more than 35°C for periods greater than 30 hours however resulted in injury to embryonic tissue. Whilst such a treatment may well induce germination, it is unlikely to occur in natural conditions in Scotland. Haas (1998) has suggested that germination occurs at a temperature of around 19°C in the second half of June, in the Swiss Alps.

Fluctuating temperatures representing day and night time temperatures have often been reported to stimulate germination in studies on aquatic plants. Arts & van der Heijden (1990), Baskin *et al.* (1996) and Probert & Brenchley (1999) found that fluctuating day night temperatures affected germination in *Littorella uniflora, Hottonia inflata* and *Zostera marina* respectively, all of these are species that can grow in shallow water. Although such conditions represent terrestrial systems and shallow water systems it may not be as representative of deep water systems, where the water acts as a buffer to temperature fluctuations. At a depth of 2m, daily water temperature fluctuations do not exceed 0.5°C, whereas at the surface they can fluctuate as much as 10°C (Frankland *et al.*, 1987). Fluctuations do not have to be large to elicit a germination response, a fluctuation of as little as 1°C can result in a 50% germination response in *Typha latifolia* (Thompson *et al.*, 1977). It has been suggested that a prerequisite of fluctuating temperatures ensures germination does not occur in water of too great a depth for plant growth (Frankland *et al.*,

1987).

6.1.2.6 Desiccation

Many species that grow in fluctuating water levels such as those found in the littoral zones of lakes, require seed drying before germination occurs. Arts & van der Heijden (1990) found this to be the case for *Littorella uniflora*. Abernethy & Willby, (1999) found drying stimulated germination in a number of flood plain aquatic plants. *Najas flexilis* forms part of the littoral community, particularly in American lakes and is not affected by winter drawdown, which may expose *Najas flexilis* seeds (Tazik *et al.*, 1982). However, *Najas flexilis* does not appear to withstand desiccation well (Hay & Muir, 2000; Muenscher, 1936). Hay & Muir (2000) classify *Najas flexilis* seeds as partially desiccation tolerant, with seeds more likely to withstand some drying before a cold treatment rather than post cold treatment. A desiccation treatment has not been reported to enhance germination.

6.2 Methods

Brown ripe seeds were collected from Loch na Cuithe Moire in September 1998, by Dr. Fiona Hay of Wakehurst Botanic Gardens. These seeds were then stored at 6 °C until November 1998. A selection of 10 seeds underwent the tetrazolium test to check for viability. Naked embryos were treated with 2,3,5-triphenyltetrazolium chloride and then incubated at 26 °C for 48 hours in the dark. Seeds were considered viable if after this procedure they were stained dark red, which they were (Bone & Probert, 1994). As this test for viability is destructive it cannot be carried out on seeds used in the experiments, therefore the results must be extrapolated to the other seeds collected and stored in the same conditions. Thus, positive results suggest viability should not be a problem.

6.2.1 Experiment 1

In order to determine the effects environmental factors have on *Najas flexilis* germination a factorial experiment was conducted. Twenty intact seeds were placed in 9 cm Sterelin petri dishes in distilled water for germination. The treatments applied to these seeds were; +/- oxygenation and +/- light at 4 different temperature regimes, 11°C, 16°C and to replicate a diurnal temperature fluctuation, 11/21°C and 6/16°C. Deoxygenated conditions were obtained by using preboiled distilled water (1.5 mg l^{-1} O_2 at 22 °C). Petri dishes were then placed in 2.5 litre AnaeroJars with an AnaeroGen sachet (Oxoid Ltd., Hampshire, England).

Oxygenated conditions were obtained by using plain distilled water (9.4 mg l^{-1} O_2 at 22 °C) and placed in identical AnaeroJars but without the sachet, these jars were opened weekly to maintain aerobic conditions. Jars were incubated in controlled temperature incubators. Dark conditions were provided by wrapping the jars in aluminium foil. Germination was recorded when the radicle had emerged. This was a similar design as used by Bone and Probert (1994) for *Najas marina*. This experimental work was carried out in collaboration with Fiona Hay at Wakehurst RBG. χ^2 tests were carried out on this data to assess whether the observed frequency of germination after 35 days was homogeneous across treatments.

These results are put into perspective by comparing them with actual temperatures recorded in lakes known to support *Najas flexilis*. The water temperature data was collected repeatedly over four years. The mean temperature for each month was calculated and this along with the standard error is shown in Fig. 6.3. This data is supplied by the Scottish Environmental Protection Agency (SEPA). It was not collected for all months of the year or on more than one occasion in each month and does not coincide with observations on *Najas flexilis*. This data therefore can only be used to suggest realistic temperatures at different *Najas flexilis* growth stages that have been observed on separate occasions in these lakes and should therefore be viewed cautiously.

6.2.2 Experiment 2

The effect of burial by sedimentation on the germination of seeds was examined. Four containers 10 cm high were filled with silty sediment, collected from the site where *Najas flexilis* grows in the Lake of Menteith (grid reference NN 577002). A strip of dialysis tubing was separated into twelve 1cm sections using bag ties. In each of these sections 5 *Najas flexilis* seeds were placed. The dialysis tubing was used as an inert seed holder that would confine seeds to maintain their position and allow easy retrieval, but allow the flow of nutrients and water. These seed holders were then placed in the containers vertically so that one section was above the water surface, another was at the water-sediment surface, and the remaining ten progressively beneath the sediment water surface. This follows the same method as Hartleb *et al.* (1993) used for *Myriophyllum spicatum*. The containers were then placed in a 32 litre black plastic tank containing approximately 25 litres of water. This was placed in a growth cabinet with a 14 hour light, 10 hour dark cycle, which was maintained at approximately 15°C. After 2 weeks the seeds were removed and the quantity of germinated seeds recorded. A χ^2 test was carried out on this data to assess whether the

observed frequencies of germination are homogeneous at different depths (Fowler *et al.*, 1998).

6.3 Results

6.3.1 Experiment 1

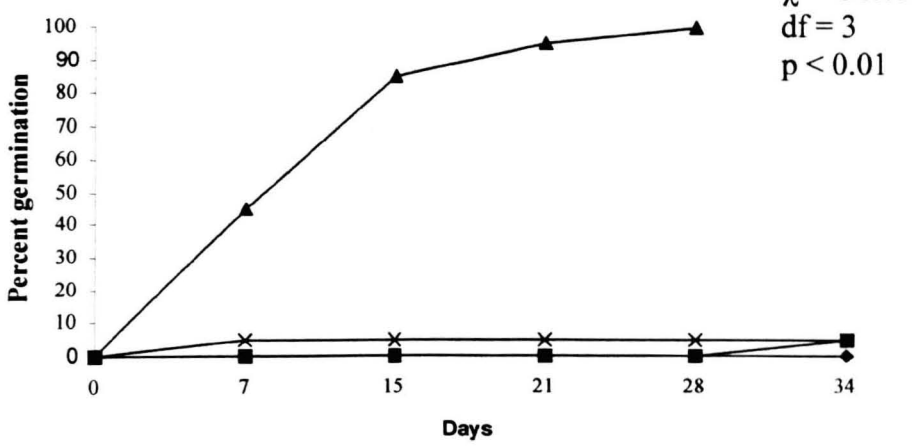
Fig. 6.1 shows the effect of the four treatments at different temperature regimes. Deoxygenated light conditions produced the highest frequency of germinated seeds at all temperature regimes except 11°C. The difference in germination results across these 4 treatments of deoxygenated dark, deoxygenated light, oxygenated light and oxygenated dark under the three temperature regimes of 16°C and fluctuating temperatures of 6,16°C and 11,21°C could not be expected by chance. At 11°C, deoxygenated dark conditions produced the highest frequency of germination after 34 days. However, the differences observed across the four treatments at this temperature regime could be expected by chance alone.

Fig. 6.2 shows the effect of temperature on the four different treatments. The differences in germination frequencies under the four different temperature regimes in oxygenated light and deoxygenated light conditions could be expected to occur by chance. In the dark deoxygenated conditions, the lowest temperature regime of 11°C produced the greatest frequency of germination. The difference in germination response to temperature in these conditions is not expected to occur by chance. The cooler temperature of 11°C also produced the greatest frequency of germination in the oxygenated dark treatment. The differences in germination in response to temperature would again not be expected by chance alone, but only at the $p<0.05$ level rather than $P<0.01$ as in the deoxygenated dark treatment.

Light deoxygenated conditions produced high levels of germination under all temperature regimes unlike the other environmental conditions. The highest temperature regime produced the greatest initial rate of germination in the deoxygenated dark conditions. High levels of germination were also observed in the deoxygenated dark conditions, but only under specific cooler temperatures. In dark conditions, the cooler temperatures produce higher levels of germination. Fluctuating temperatures were not found to produce a significantly greater germination response than a constant temperature.

Stratification had been planned as an additional treatment to those investigated. However, any stratification requirements by the seeds appear to have been satisfied by the 2 month period at 6°C, which all seeds in this experiment were kept at for storage before

a)



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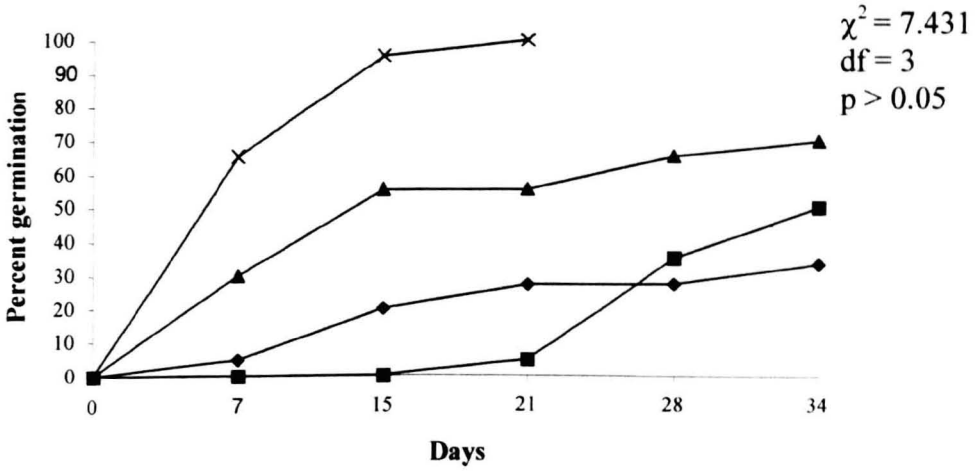

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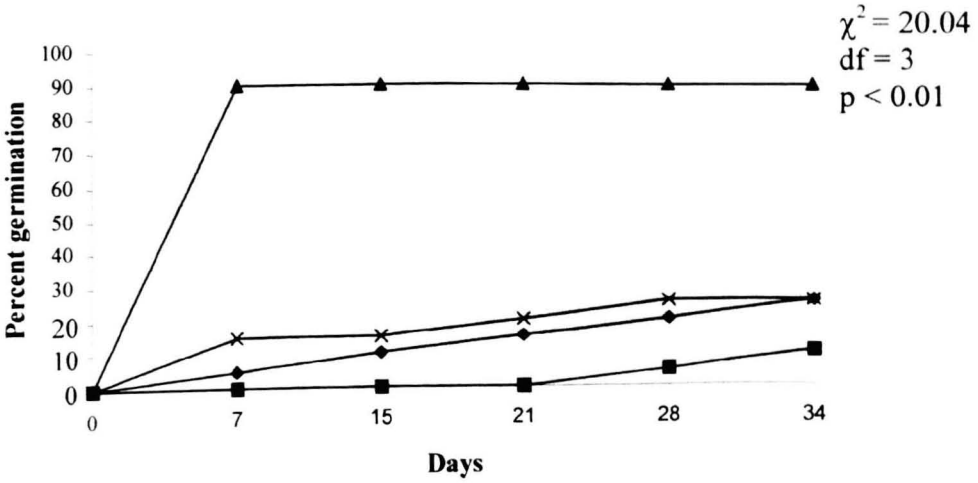

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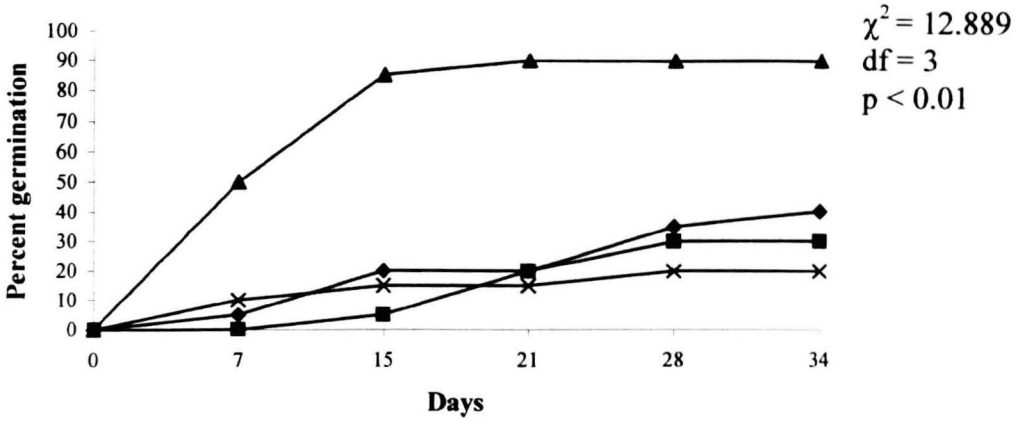
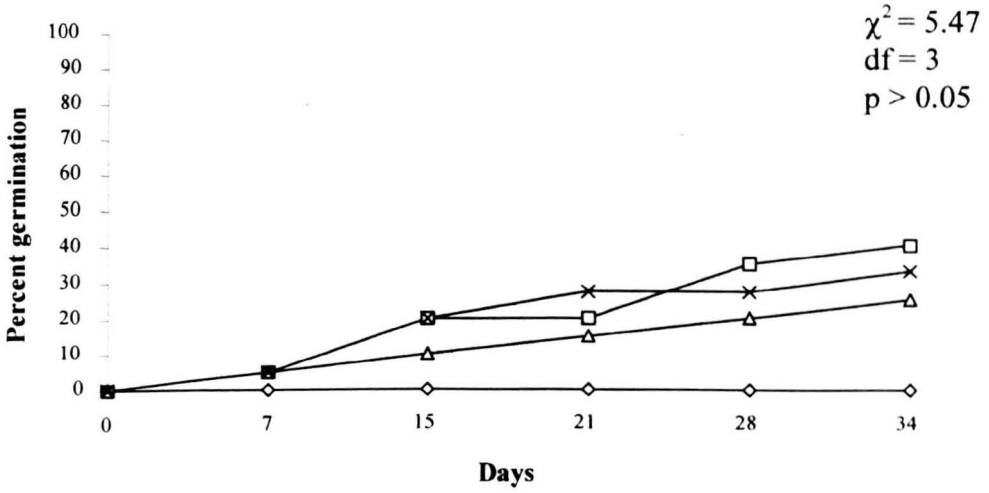


Fig. 6.1 Percentage germination at a) 16° C, b) 11° C, c) 11/21 ° C and d) 6/16° C under different conditions. Where; ◆ = + O_2, + light, ■ = + O_2, - light, ▲ = - O_2, + light, × = - O_2, - light. χ^2 is calculated on the number of seeds germinated after 34 days.

a)



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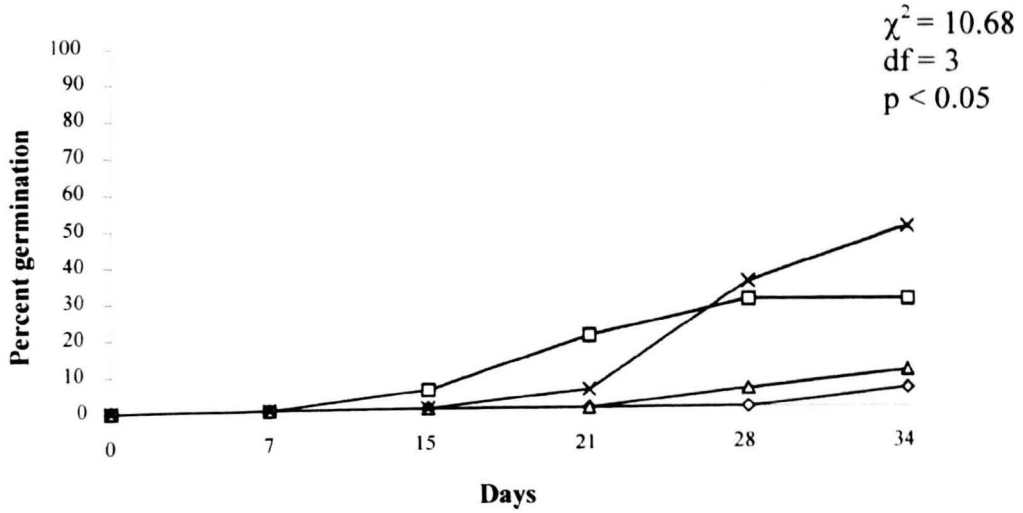

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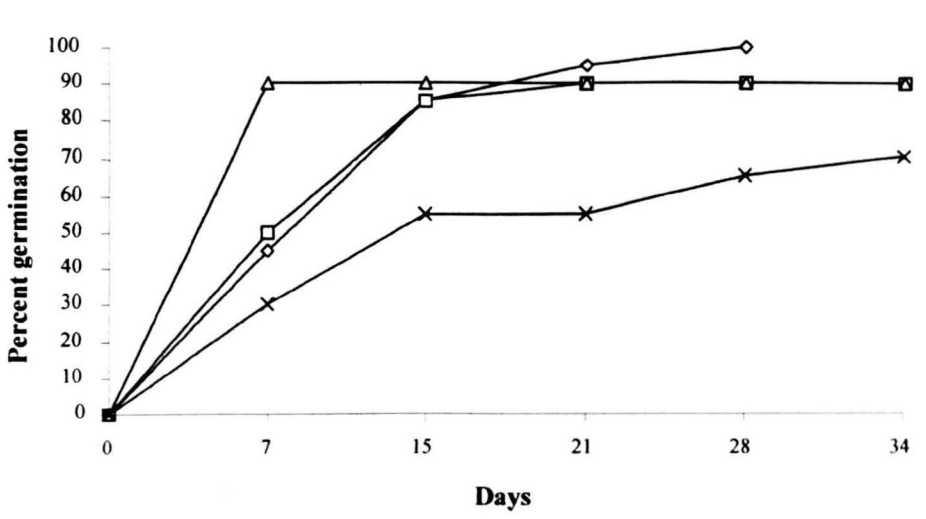


d)

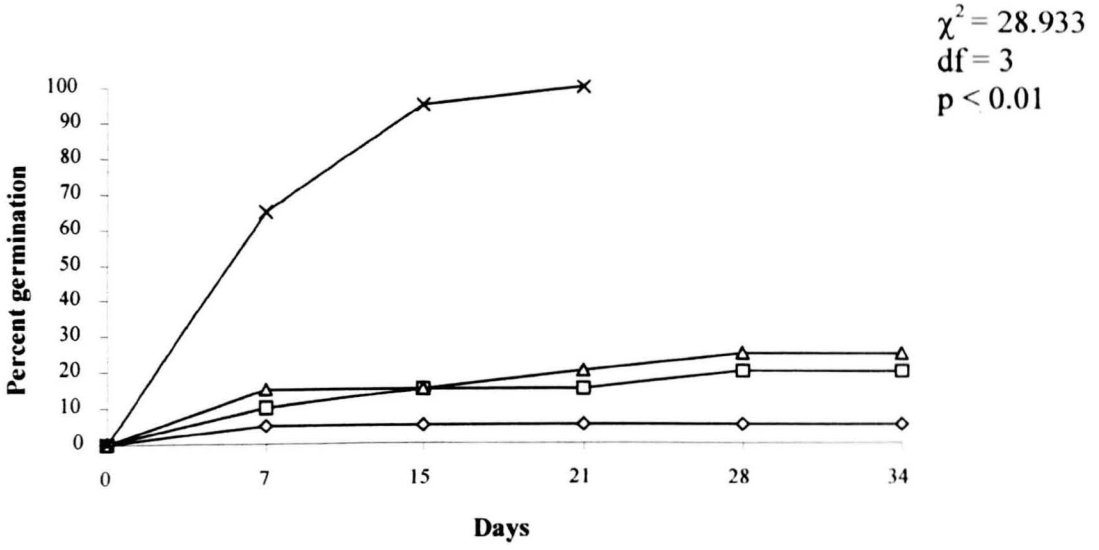


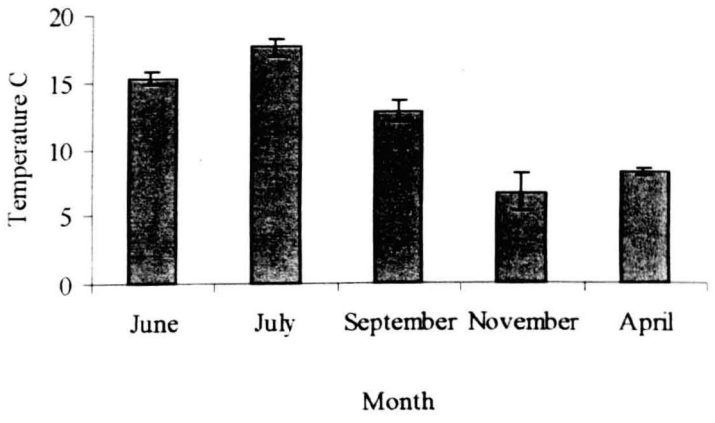
Fig. 6.2 The effect of temperature on germination rate for each treatment where; a) = $+O_2$, + light, b) = + O_2, - light, c) = - O_2, + light, d) = - O_2, - light and △ = 11, 21°C, □ = 6, 16°C, ◇ = 16°C, × 11°C. χ^2 is calculated on the number of seeds germinated after 34 days.

experimentation. The high levels of germination success also suggested that there was no physical dormancy to be broken by scarification as in *Najas marina*.

In Scotland germinating *Najas flexilis* plants have been observed in June, they grow throughout July and August and by September ripe brown seeds can be seen on the plants (personal observation & Valerie James pers comm.). When this phenology is compared to the temperatures recorded on separate occasions in lakes supporting *Najas flexilis* (Fig. 6.3) it can be suggested that germination would occur when lake temperatures are approximately between 11 and 16°C. Growth would continue as the temperature rises. The highest temperature recorded in these lakes by SEPA is 20°C, which is much cooler than the 35°C,

which promoted germination in the study by Wetzel and Mcgregor (1968). Ripe seeds are produced when temperatures are approximately 10-14°C. This temperature range is not much lower than that observed when germination would occur. This suggests that some form of mechanism would be required to prevent immediate germination, such as a stratification requirement. November and April temperatures do not always drop below 6°C, the stratification temperature used in this experiment, however these are not the coldest months of the year in Scotland. Unfortunately, there is no consistent data for the colder months of the year. It can be presumed that temperatures would consistently reach below 6°C in the winter, as the temperature is almost that low in the months preceding and following the colder months. Although temperatures rise to approximately 8°C in April, germination does not occur until June when temperatures are above 10°C. These temperatures correspond to those seen to promote germination in the previous experiment

a)



b)

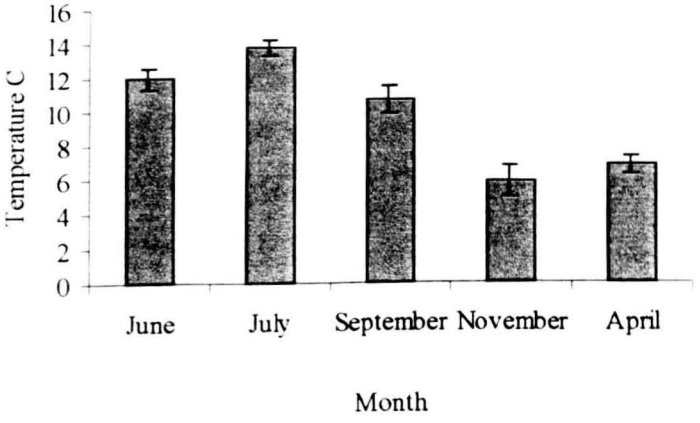


Fig. 6.3 Average monthly water temperatures in a) Loch Butterstone and b) Loch Craiglush both lochs containing *Najas flexilis*. Average temperature is calculated from once monthly measurements from the 4 years 1993-1996 inclusively (Data supplied by the Scottish Environmental Protection Agency).

6.3.2 Experiment 2

The greatest quantity of seeds germinated at the sediment-water surface, however seeds also consistently germinated regardless of depth. These results can be seen in Fig. 6.4. A χ^2 test shows that the observed frequencies are homogeneous and the departure is merely due to sampling error or scatter. This shows that burial alone does not effect seed germination. However germination only reached a maximum of 50% after two weeks, this is less than in the previous experiment suggesting that some other factor may also be inhibiting germination in this experimental set up. The sediment was left in pots for only 2 weeks before the experiment began. In this time it is unlikely that the environmental gradients usually found with depth in the sediment would have established, for example decreasing oxygen concentration with depth. However, the previous experiments have already indicated that deoxygenated conditions promote *Najas flexilis* germination. It is also of note that a similar experiment on *Myriophyllum spicatum* found a significant decrease in germination with depth when again the sediment had not been left for a prolonged time to establish such environmental gradients (Hartleb *et al.*, 1993). It is difficult to draw conclusions from this experiment because the depth at which the seed is found in the sediment in the lake environment may have a different effect to that observed in this experiment due to established environmental gradients in the lake sediment.

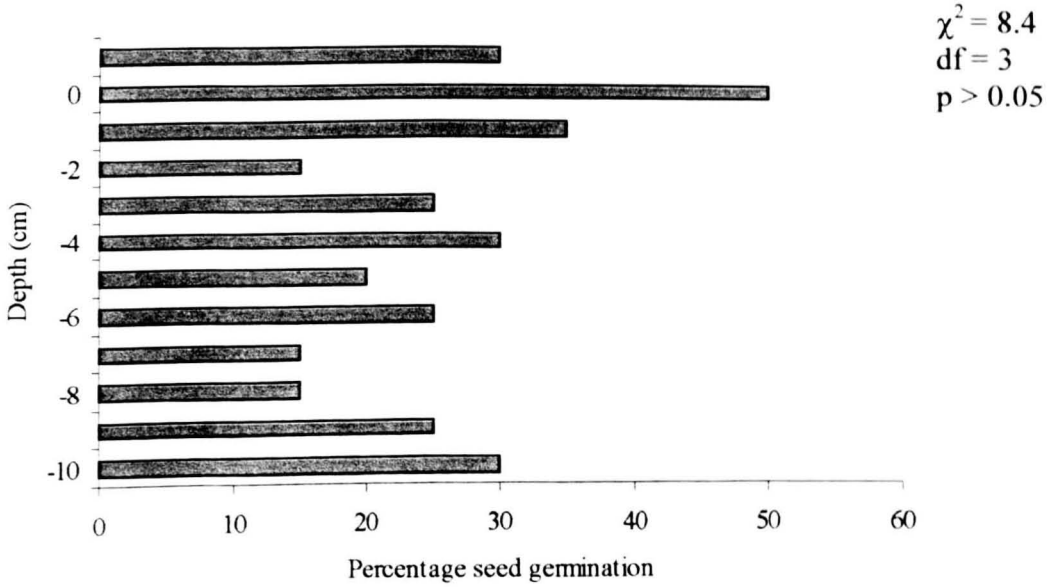


Fig. 6.4 Percent germination of *Najas flexilis* seeds at varying depth in the sediment. χ^2 is calculated from the total number of seeds germinated after 2 weeks

6.4.1 Temperature

Germination does not occur at 6°C, this was observed when seeds were stored at this temperature before germination. Therefore, warmer temperatures are required to stimulate germination. 11°C appears to be sufficient to stimulate germination. Although warmer temperatures appear to stimulate greater quantities of germination in light, deoxygenated conditions, there is no significant difference between the responses to these temperatures in these conditions. Light and warmth stimulating germination is typical of a summer annual such as *Najas flexilis*. However, in dark, deoxygenated conditions the cooler temperature of 11°C appears to stimulate greater quantities of germination than the higher temperatures. It is probable that in the shallow waters where *Najas flexilis* grows it will germinate in response to the warmer light conditions. Where *Najas flexilis* grows in deeper water, it is most likely that germination will occur in response to the cooler temperature of 11°C and darker conditions. A fluctuating temperature did not appear to be required. As *Najas flexilis* can grow at depths of up to 14m this is not surprising as the water will act as a buffer to temperature fluctuations.

6.4.2 Light/dark

In contrast to the findings of Wetzel & McGregor (1968) it appears that light did not inhibit germination, and that the best results, for all temperature regimes, were gained under deoxygenated, light conditions. However under the right conditions, i.e. 11°C and deoxygenated, germination can also occur in the dark. Fieldwork measurements and observations (see Chapter 2) have shown that *Najas flexilis* can grow in waters of varying clarities, at varying depths, which would suggest that a tolerance of both light and darker conditions would be advantageous. The ability of *Najas flexilis* seeds to germinate in the dark was further emphasised by the consistent germination of seeds at depth in the sediment. The ability to germinate after burial under a limited amount of sediment could be seen as an advantage, yet germinating under 10cm of sediment would appear a waste if seedlings could not reach the surface. The greatest depth of sediment through which *Najas flexilis* can germinate, grow and survive is unknown and requires further experimentation.

Tolerance of completely deoxygenated conditions is not unusual in aquatics and has been found in rice *Oryza sativa,* and barnyard grass *Echinocloa crus-galli* (Baskin & Baskin, 1998). Other aquatics have been found to germinate to high percentages only under low oxygen conditions. Examples are *Typha latifolia* (Bonnewell *et al.*, 1983), *Hydrilla verticillata* (Lal & Gopal, 1993) and *Potamogeton schweinfurthii* and *P. richardi* (Spence *et al.*, 1971). This preference for low oxygen levels is important for plants that grow in temporary aquatic systems, as it ensures that seeds germinate when the sediment is flooded. A preference for deoxygenated conditions in constantly aquatic systems has been suggested to ensure that seedlings grow in stable environments.

High oxygen levels in the water or sediment will be predominantly due to mixing, which is a form of disturbance to seedlings. Seedlings germinating where oxygen levels are higher, in the water column, on the sediment surface or in the top oxygenated microzone of the sediment, are also those that are most likely to be disturbed by wave action before a sufficient anchorage system can be grown. Germination in the deoxygenated sediment therefore permits the establishment of an effective root anchorage system and an immediate access to a supply of nutrients, which would promote successful seedling establishment (Moore *et al.*, 1993).

6.5 Conclusion

Knowledge of the germination requirements of *Najas flexilis* helps elucidate the phenology of *Najas flexilis* establishment and growth in Scotland. This illustrates that the timing of any seed reintroduction programme would be important, especially if the lake water clarity was low. This is because the temperature window for optimal germination is lower in dark conditions. A cold pre-treatment for seeds before reintroduction would be advisable. This could be gained by introducing seeds to the lake in autumn so that they experience the natural winter conditions. However, a greater time spent in the lake before germination increases the chance of predation or burial. Therefore, it is probably safer to keep seeds in water at 6°C before reintroduction in the spring, as 6°C appears to satisfy any stratification requirement of the seeds. The preference for deoxygenated conditions is most likely to be met in relatively stable substrates. This agrees with the findings of Chapter 2, which illustrates that *Najas flexilis* often grows in the sheltered parts of lakes. If the previous

situation of the plant within a lake was unknown, these findings further suggest that a sheltered area would appear to be an appropriate site for reintroduction.

Summary

- *Najas flexilis* germination is greatest in light deoxygenated conditions at warm temperatures (temperatures of 16°C is sufficient).
- If dark conditions prevail germination is greater at cooler temperatures (11°C).
- These conditions can be found in Scottish lochs in sheltered areas in June.
- Burial of seeds in lake sediment to a depth of 10cm did not appear to affect germination, although the high percentages of germination observed in the previous experiments were repeated in this experiment.

7. Discussion

7.1 Summary of the results

7.1.1 Chapter 2

Chapter 2 considers the relationship between *Najas flexilis* and the environment. It shows that measurement of specific plant traits; leaf area and reproductive number, from a representative sample of the population is a possible method for assessing the condition of the population in response to the environment. This is important because accurately measuring plant abundance to assess the condition of the population in deep water plant communities is extremely difficult due to methodological difficulties.

This chapter also found that the pH, conductivity, alkalinity, calcium, magnesium, total phosphate and potassium concentrations in the water were significantly different in sites where *Najas flexilis* is extinct compared to sites where it thrives today. It also found that the available phosphate and iron in the sediment are significantly different at present and absent sites. Models were also produced which found that the concentration of phosphate in the sediment and water and magnesium in the water, along with the alkalinity and either light or calcium in the sediment could predict 67 - 88% of the variation in the plant traits of *Najas flexilis*, depending on the traits and the environmental variables included. Chapter 2 also shows how exposure affects the distribution of *Najas flexilis* within a lake and how there is a trend between *Najas flexilis* distribution and light, but improved methodologies might find this effect significant. There was no significant relationship between macrophyte biomass and *Najas flexilis* plant traits.

7.1.2 Chapter 3

Chapter 3 compares a small-scale quantitative approach to a lake scale qualitative approach of plant community classification, to determine their abilities of describing the lake environment. The lake scale qualitative approach elucidated significant differences in pH, alkalinity and the concentration of total oxidised nitrogen and dissolved reactive phosphate in the water. It also elucidated differences in phosphate, calcium and iron in the sediment. In contrast, the small-scale quantitative approach only elucidated a difference in light. The community groups attained from the small-scale quantitative survey were roughly comparable to NVC communities A13, A24a and A23. The lake scale qualitative approach produced community groups comparable to standing water type 3, 4 and 5. However, this

study produced two type 5 sub-groups. Extinct *Najas flexilis* sites were classified as type 3 and the most eutrophic type 5, as were some other sites that could still support *Najas flexilis*. No extinct sites were classified as type 4. This shows that although standing water type can detect which sites may be more at risk and allow resources to be concentrated on those sites, it can not pinpoint extinct and present sites.

7.1.3 Chapter 4

Chapter 4 looks at the plant attributes present within a macrophyte community, which may confer a competitive advantage over *Najas flexilis*. This is compared to the possible threat from the alien invasive *Elodea* spp. and the attributes these species possess. A method for calculating the quantity of plant attributes within a macrophyte community is described and the relationship these attributes have with *Najas flexilis* is calculated. The resultant model, which includes the quantity of plants in the community possessing the following attributes; wind pollination, June-July flowering and being a perennial, explains 68% of the variation in *Najas flexilis* leaf area/shoot length. Both *Elodea* spp. are perennials and flower in June-July, but are epihydrophilous and not wind pollinated.

The possible reasons for the importance of these traits are that early flowering plants not only flower before *Najas flexilis*, but also begin to grow before *Najas flexilis* thus obtaining resources before *Najas flexilis* can compete for them and then shading *Najas flexilis* with its early growth. The perennial plants will have a greater quantity of stored resources to facilitate growth than *Najas flexilis* seeds hence providing perennial plants with a head start in any competition with the annual *Najas flexilis*. The benefit of being a wind pollinated plant in competition with the hydrophilous *Najas flexilis* maybe the reliability of pollination, but is also possibly due to the fact that wind pollinated plants must grow to the water surface to support flowers above the water surface, these taller plants will then potentially shade *Najas flexilis*.

This work indicates that a proliferation of invasive or native plants with these traits within the *Najas flexilis* micro-habitat can potentially have a deleterious effect on the *Najas flexilis* populations. However, a proliferation of a native species is most likely to be due to an environmental change such as the increase in a previously limiting resource such as phosphate. Therefore the cause of any weed problem must be considered as well as the weed itself. Monitoring invasive plants would indicate when or if invasive species cause a

threat to *Najas flexilis*. In order to protect the species if invasive species such as *Elodea* spp. is a threat would require action such as the removal of the invasive species or saving the genotype until conditions are more favourable for *Najas flexilis* growth possibly in the millennium seedbank.

7.1.4 Chapter 5

Chapter 5 looks at the genetic diversity within the Scottish and Irish populations of *Najas flexilis* using RAPDs. The division of diversity within and between populations is compared to what would be expected according to current theory relating genetic diversity to life history characteristics. The data suggests that there is no great division of diversity between Scottish and Irish populations. The individuals appear to be more similar to other individuals within the same waterbody, rather than individuals in other waterbodies. All but one pair of individuals appear to be genetically unique, suggesting that selfing is not the predominant form of pollination and that each population is relatively isolated with little movement of seed or pollen between lakes.

The frequency of RAPD bands within a population is compared to the environment of that site and the traits of *Najas flexilis* at that site and no significant relationship is identified. A significant relationship however is identified between exposure and diversity within a population. This is most likely to be because increased water movement caused by exposure also moves pollen and seed making it less likely that a plant will be self pollinated or will cross pollinate with a sibling plant. This is because seeds from the same parent plant are dispersed within the lake so that a plant's nearest neighbour is less likely to be a sibling.

7.1.5 Chapter 6

Chapter 6 looks at the germination requirements for *Najas flexilis* and identifies that germination can occur at temperatures above 11°C, which is comparable with reports of seeds germinating in June in Scotland. The best germination results were achieved in warm (16°C), deoxygenated, light conditions. However, if dark conditions prevailed greater germination occurred at 11°C. Further experiments found that there was no significant relationship between seed depth within the sediment and germination response.

7.2 What are the implications for the conservation of *Najas flexilis?*

7.2.1 Threats to the species

It appears from this research that *Najas flexilis* occupies a relatively small realised niche in Britain and Ireland. This is illustrated by the fact that a single standing water type can describe communities that do and do not support *Najas flexilis.* Therefore, whilst other macrophytes can exist over a wider variety of environmental conditions the environment that can support *Najas flexilis* is more specific. If a DOME score (Palmer, 1989) were to be assigned to this species it would be 'om' as it is present in some, but not all oligotrophic sites (SWT 2 & 3) and some but not all mesotrophic sites (SWT 5). The only other species with this DOME score assigned to it is *Pilularia globulifera,* another rare aquatic species. This narrow realised niche means that compared to other aquatic plants which are more ubiquitous there are fewer environments to support *Najas flexilis* and the environments are more likely to become unsuitable for *Najas flexilis* growth as small changes in the environment can have a detrimental impact. There may be more sites where this species grows than is presently recorded, as the plant grows in deep unclear water. In sites where it occurs it is not necessarily widespread throughout the lake. It is not easily caught on grapnels, and has a relatively short growth season, so if the site was not surveyed in August or September it is unlikely to be found.

It is also likely that this plant is under recorded in Britain and Ireland along with other aquatic plants of a similar habit such as *Elatine hexandra.* Both these plants can grow in deep water never reaching the surface, thus keeping them from view of the more terrestrial botanist. Neither of these plants float, this means that they are less likely to be found in the strandline. *Najas flexilis* can be found in the strandline, but only in lakes where the slope of the lake bed is not steeply sloping or rocky as the plant then gets trapped and does not make it to the shore. *Najas flexilis* is never found in the shoreline as abundantly as other floating plants such as *Elodea* spp. or *Littorella uniflora.* This may also be related to the fact that it is rarely as abundant as these two species within a lake. This patchy distribution within a lake may also be a factor in its under recording. Even a keen aquatic botanist searching from a boat or swimming in the water could believe they have recorded all the plants species within a lake when they are yet to find the small single patch of *Najas flexilis.* The other two factors which lead to the under recording of *Najas flexilis* is its short life cycle and the fact that the standard tool for finding aquatic plants, the grapnel, does not work particularly well at sampling *Najas flexilis.* This is due to its slender form, which allows it to escape the

grapnel and the fact that it can easily break and not be entangled in the grapnels hooks. However, in deep unclear waters this is often the only tool available to the aquatic botanist to find the plant without expensive scuba diving or video recording. Although the success of identifying plant species from videotape can be questionable.

7.2.1.1 Eutrophication

As *Najas flexilis* thrives only in mesotrophic conditions, it is of no surprise that eutrophication is a threat to the species. However, light is not always the limiting factor and clear water is not a prerequisite for *Najas flexilis* growth. It has been reported here (Chapter 2 & 3) and elsewhere in the literature (e.g. Hough & Fornwall, 1988) that *Najas flexilis* can grow in low light conditions and often grows in deep water, occupying the same area of a lake as *Nitella* spp. (Pearsall, 1920). Instead, the mechanism via which *Najas flexilis* is unsuccessful in such sites where eutrophication has occurred appears to be its inability to photosynthesise, due to a lack of available CO_2. Indicators of such conditions are high pH, alkalinity and phosphorus. These can all be seen in Loch Marlee in Perthshire from which *Najas flexilis* recently appears to have become extinct.

7.2.1.2 Acidification

Acidification is another threat to *Najas flexilis.* The effect of such conditions on *Najas flexilis* was much harder to quantify. This is because in such conditions the plant becomes even more elusive. This is probably due to decreasing reproductive success in such conditions. In Lough Pollacapull and Lough Shennagh, a long time was spent searching for the plant using both snorkelling and shoreline approaches. In these situations after a frustrating search, fragments were found floating in the water with no seeds attached. The other more acidic sites surveyed, where whole plants were found, also had low seed numbers, particularly the plants found at Roundstone Lough. The mechanism via which seed production is limited is unknown, and this would require further studies to elucidate. However, these observations of reduced reproductive capacity in more acidic conditions agree with experimental results in the literature (Titus & Hoover, 1991; Titus & Hoover, 1993).

7.2.1.3 Weed growth and competition

The effect of competition from other macrophytes particularly the alien invasives *Elodea canadensis* and *Elodea nuttallii* was difficult to determine. This is because the competitive effect of the *Elodea* spp. would depend on the time since introduction and the

environmental condition of the lake. It would appear from the literature that *Elodea* spp. are most likely to be a threat in situations where the lakes are small and shallow (Pearsall, 1920; Sheldon & Boylen, 1975; Pip & Simmons, 1986; Hough & Fornwall, 1988; Simpson, 1988; Spicer & Catling, 1988; Scott, 1996). This is because there are no deep areas in the lake, where light levels may be too low for *Elodea* spp. to grow, but *Najas flexilis* can survive. This situation will be exacerbated by higher nutrient levels, as the literature (Grime *et al.*, 1988; Simpson 1990; Best *et al.*, 1996; Preston & Croft, 1997) suggests that the niches of *Elodea* spp. and *Najas flexilis* overlap in the more nutrient poor sites where *Elodea* spp. grow, which are the more nutrient rich sites where *Najas flexilis* grows. This information from the literature agrees with observational data from this study. The site where *Najas flexilis* has been reported to be limited by *Elodea canadensis* growth is Tangy Loch. This loch is small and shallow in comparison to the large lakes in Perthshire and Stirlingshire where both *Najas flexilis* and *Elodea* spp. grow without obvious detrimental effects. However, this observational evidence is confused by the fact that these mainland sites were invaded by *Elodea* spp. before the smaller Tangy Loch. It may be that time since invasion is the factor that affects the ability for *Elodea* spp. and *Najas flexilis* to coexist, and that lake size is coincidental.

It is worth reiterating that only one site, Tangy Loch, has been reported where *Elodea canadensis* was thought to be detrimentally affecting *Najas flexilis* growth. The only site surveyed in this study where excessive weed growth appeared to be detrimentally affecting *Najas flexilis* was Loch nam Cnamh. In this case, the plant species in question were *Myriophyllum alterniflorum* and *Chara* spp. The biomass of these two species combined from Ekman samples was greatly in excess of any other sample collected. We were told by the crofter that this site was 'Loch of the Bones' and that people who could not be buried in the nearby churchyard, had in the past been put into the loch. Therefore, the excessive weed growth may well have been due to nutrient enrichment from this source although further studies would be required to confirm where the nutrients were coming from. Chapter 4 suggests plant traits that may make an aggressive competitor for *Najas flexilis.* Chapter 4 also provides a list of species, which possess these traits and grow in the same environment as *Najas flexilis. Elodea* spp. are not included in this list, but *Myriophyllum alterniflorum* is included.

The relationship between *Najas flexilis* and other macrophytes is not clear. Observational evidence suggests that competition can be a problem, but is not always so. The situations

where aquatic weed growth could be a threat are likely to be sites where there is sufficient nutrients and light to enable excessive plant growth. It is suggested that the *Elodea* situation in the Outer Hebrides and its effect on *Najas flexilis* is monitored over the forthcoming years to help clarify the situation. However in order to ensure the success of *Najas flexilis* action would need to be taken if monitoring found *Najas flexilis* to be threatened. This may take the form of removing competing macrophytes or conserving the genotype of the lake as seed until it can be reintroduced when conditions are improved.

The distribution of *Najas flexilis* may appear to be increasing as more sites are searched for the presence of this plant and the plant is found. However there are also a number of sites where the plant is believed to be extinct. These appear to be due to acidification and eutrophication. The reversal of this state is a question for the statutory government agencies. Before reintroduction is considered the sites must first be suitable for *Najas flexilis* growth. Due to logistical reasons only the mainland sites with only pre-1980 sites were visited. The sites where *Najas flexilis* was not found were in Perthshire and Fife, the more intensively agricultural areas where *Najas flexilis* can be found. All these sites appear to be more eutrophic than those that support *Najas flexilis*. It is likely that the eutrophication has occurred through diffuse agricultural sources in the catchment. Fish farms are mentioned in the Biodiversity Action Plan as a possible threat to *Najas flexilis*, yet sites with fish farms (Loch Butterstone and Lake of Menteith) successfully support *Najas flexilis* populations of reasonable fitness. Therefore if *Najas flexilis* is to be reintroduced to extinct Scottish sites the source of nutrients into the lakes need to be confirmed and reduced first. These are most likely to occur through agri-environmental schemes if the main source of nutrients is from agriculture.

7.2.1.4 Climate change

Whilst some of the British flora appears to be rare in Britain as it is a relict from a previous climate (such as the more alpine element of the British flora, which is a relict of a colder climate) this does not appear to be the case with *Najas flexilis*. The climatic relict populations often do not thrive where they grow in Britain and often do not reproduce successfully. In contrast *Najas flexilis*, where it can be found, grows in abundance and reproduces readily (sufficiently to maintain its populations, as it is an annual). It is also of note that in Europe *Najas flexilis* can also be found further north than the Scottish populations in Finland and further south in Germany and Poland, again suggesting that *Najas flexilis* is not situated at its climatic limits in Scotland (Hultén & Fries, 1986). The

Southern Irish sites in Kerry maybe close to the warmest climate *Najas flexilis* inhabits in Europe. There are more southerly *Najas flexilis* sites in Europe in the Alps, but it can be assumed these sites will be colder due to altitude. The North American sites also occur at more southerly latitudes, but the climate is colder in the winter and warmer in the summer at these sites due to a continental climate and lack of the Gulf Stream warming the continent.

This study did not investigate the effect of temperature on *Najas flexilis* directly except with regard to germination. This work shows that cold temperatures (approximately 6°C) are required in winter so that seeds can germinate in the spring. As an annual *Najas flexilis* requires summer temperatures of approximately 11°C to germinate and for these temperatures to be sustained for long enough for the plant to produce ripe seed, approximately three months. This together with the geographic distribution of *Najas flexilis* suggests that relatively minor shifts in temperature, either warmer or colder, would not threaten the Scottish *Najas flexilis* populations.

7.2.2 Monitoring

As discussed in Chapter 3 EC member states have to report on the implementation of measures taken under the Habitats Directive every six years. That includes the 'surveillance' of the conservation status of certain species, including *Najas flexilis*. The UK conservation agencies are also currently drawing up 'site condition monitoring protocols for SSSIs, which will be applicable to many *Najas flexilis* sites. Monitoring is important for both sites where the plant is present and sites where the aim is to reintroduce the species. Monitoring the present sites should provide a warning system if sites become unfavourable for *Najas flexilis* growth. Monitoring of potential sites for reintroduction should provide information on when a site is in a condition where reintroduction stands a chance of success. As discussed in Chapter 3, the factors being monitored do not have to be *Najas flexilis* itself, but indicators of its status. This study has produced three possible monitoring methods; monitoring *Najas flexilis* itself using plant traits such as leaf area and shoot length, monitoring the plant community present, monitoring water and sediment chemistry.

7.2.2.1 Approach 1; *Najas flexilis* plant traits

Chapter 2 describes the use of traits to assess plant fitness. This is a useful alternative approach to abundance measures in aquatic systems, where water clarity can cause problems for accurately assessing abundance. This would be a suitable monitoring method

for present sites. The variation in the plant fitness value log (leaf area/shoot length) x (number of reproductive structures/shoot length) is shown in Fig. 2.3. It can be seen that there is a step increase between those sites with a value of less than one, and the rest of the sites. If sites have a score of less than one it would seem sensible to be concerned about the future of these sites. The sites found to have scores of less than one, in this study, had so few seeds it would be unlikely that any progeny would survive. It was also these sites where few individuals were found. This monitoring method would involve snorkelling or boat surveys, as plants cannot always be reached from the shore. It would be advisable to undertake such a monitoring programme in August or September in order to find mature plants. By October, the plants are dead and in July, they are small, harder to find via grapnel surveys and have fewer mature seeds.

7.2.2.2 Approach 2; water and sediment chemistry

Chapter 2 describes the sediment and water chemistry at present and extinct sites. If the sediment and water chemistry is to be analysed for monitoring habitat condition for *Najas flexilis*, it is suggested that samples are taken at the time of *Najas flexilis* growth i.e. August or September. This is because this is when the data in this study was collected, and this is the data to which it would be compared. Many of the measurements of water and sediment chemistry overlap both the present and absent groups. This is because the factors that will affect *Najas flexilis* will interact and not affect *Najas flexilis* growth in isolation. In addition, the measurements taken at the extinct sites were not taken at the time of extinction, so environmental conditions may have changed since that time. Monitoring the water and sediment chemistry would give an indication of the site condition at both present and extinct sites, but could not be used to pinpoint when a site is suitable for *Najas flexilis* growth. The models developed in Chapter 2 would give a more precise estimate of site condition suitability for *Najas flexilis* growth. This is because these models take into account some of the interactions between the chemical factors. These models would be useful tools to predict when a site is in a suitable condition for reintroduction.

7.2.2.3 Approach 3; macrophyte community classification

Plant community type could also be used to monitor *Najas flexilis* present and absent sites. Qualitative surveys for plant communities may well be easier, less expensive and less time consuming than locating *Najas flexilis* within a lake and assessing its fitness. This is a broad scale approach and would not pinpoint at risk sites. However, it could indicate the group of

lakes most likely to include at risk sites. This is because extinct sites were only classified as standing water type 3 and the eutrophic standing water type 5. All sites classified as these standing water types, could then be surveyed for *Najas flexilis* specifically. Alternatively, a change in standing water type classification since the previous survey would also merit investigation.

The problem with using macrophytes for monitoring (approach 1 & 3) is that it is unknown how quickly macrophytes respond to a change in the environment. It is possible that by the time that a change is shown in the macrophytes the environment will have been unsuitable for a number of years. Whilst the water and sediment chemistry is more sensitive, it may be too sensitive. Measurements are unlikely to be constant over the years, as pH fluctuates daily with light availability and photosynthesis. Therefore, a change in this measure between one years sampling and the next may have little consequence for *Najas flexilis*. Therefore, it is important either to compare such chemical measures with the presence and absence chemical data (Table 2.3 a & b), or apply them to the models in Chapter 2, to ascertain if this fluctuation in water chemistry is likely to have any consequences for *Najas flexilis*.

Results of any monitoring will show sites to have a range of conditions and a range of *Najas flexilis* fitness measures. Those with lower fitness measure or conditions less than optimal for its growth will not necessarily require management action, as that is their normal state. However, if sites are changing towards an unsuitable state for *Najas flexilis* action may be required. Since the start of this study, work has been carried out at Wakehurst Botanic Gardens as part of the millennium seedbank project on methods for storing *Najas flexilis* seeds (Hay & Muir, 2000). If sites are deteriorating or *Elodea* has newly invaded a site, it may be a precautionary measure to conserve the lake's genotype by storing these seeds appropriately. Seeds are not desiccation tolerant.

7.2.3 Reintroduction

Before *Najas flexilis* can be reintroduced to any site, the lake environment has to be in a suitable condition. This can be assessed by the above monitoring schemes. Where within a lake *Najas flexilis* should be introduced is an important issue. There are not always records of the distribution of *Najas flexilis* within lakes before extinction. This is certainly the case for the extinct Perthshire sites with the exception of Loch Marlee. The relationship between *Najas flexilis*, light, depth and exposure described in Chapter 2 would help to determine possible reintroduction sites within a lake. *Najas flexilis* was only ever found in this study

on fine silty sediment, which agrees with comments in the literature (Pearsall, 1920; James & Barclay, 1996; Howson *et al.*, 1997). This explains in part the relationship between *Najas flexilis* and exposure. As a result, *Najas flexilis* is often found behind islands or peninsulas or in sheltered bays. If the lake fetch is large, *Najas flexilis* plants are most often found in deep water. However, the depth at which the plant can occur is likely to be limited by light (see chapter 2). The range of exposure and light where *Najas flexilis* was observed to grow at different depths is shown in Fig. 2.5 & 2.6. This can be used as guidelines as to where to reintroduce *Najas flexilis* within a lake. NVC community type may also be a guide as *Najas flexilis* was never observed in the *Littorella-Lobelia* community usually in abundance in the littoral zone, but in the NVC communities A13, A24a and A23 described in Chapter 3.

As *Najas flexilis* does not reproduce from vegetative fragments and is an annual with fragile above and below ground structures, introduction of seed rather plants would be the advisable method of reintroduction. The results of the germination experiments (Chapter 6) show that any seed should be introduced by May to allow germination in June. It would be advisable to keep seed in water at temperatures of 6°C or below before introduction. This would satisfy any stratification requirements and would prevent loss of viability due to the seeds intolerance to desiccation.

Where any seeds should be introduced from is an important issue and the genetic study described in Chapter 5 does not provide any easy answers. Therefore, the best approach could well be one of matching sites environmentally to find a donor site. This also obviously depends on the fitness of the population at the matched sites. Collecting seeds from populations which produce few seeds can not only be detrimental to the donor population, but it is also possible that the donor population may be suffering from inbreeding depression, thus making it an unsuitable donor site.

This study has provided information for the conservation of *Najas flexilis* in Britain and Ireland; elucidating the threats to the species, proposing possible methods for monitoring and advice on reintroduction. It was beyond the scope of this study to investigate the *Najas flexilis* seedbank and the longevity of *Najas flexilis* seeds. If viable seeds could be found in the seedbank it would be worth considering as an alternative source for reintroduction. The growth of *Elodea* spp. in North and South Uist is an issue worthy of further study. As the species invade new lakes, they should be monitored throughout their establishment phases,

in different environmental conditions, so that the risk they pose to the aquatic macrophytes of this area can be understood more clearly.

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Appendix 1

Site abbreviations

Site	Abreviation
Mainland	
Loch a' Bhada Dharaich	Bh
Loch of Butterstone	But
Loch of Clunie	Cl
Loch of Craiglush	Cr
Fingask Loch	Fin
Loch Kindar	K
Lindores Loch	Li
Loch of Lowes	Lw
Loch of Drumellie	M
Lake of Menteith	Lom
Monk Myre	Mm
Loch Monzievaird	Mo
White Loch	W
Inner Isles & Kintyre	
Loch Ballyhaugh, Coll	Ba
Loch Fada, Colonsay	Fa
Glenastle Loch, Islay	Gl
Loch Gorm, Islay	Go
Loch Lossit, Islay	L
Loch Nan Gad, Kintyre	Ng
Loch Poit na h-I, Mull	Po
Tangy Loch, Kintyre	T
Loch an t-Sagairt, Coll	Ats
Outer Isles, North & South Uist	
Loch Altabrug	A
Loch Bun an Ligidh	Bal
Loch nan Capull	Nc
Loch nan Cnamh	Cn
Loch Cuilc	Cu
Loch na Cuithe Moire	Cm
Loch Druidibeag	D
North Loch Druidibeag	Nd
Loch Eaval	E
Loch an Eilean (Drimsdale)	Ed
Loch Eilean a' Ghille-ruaidh	Egr
Loch an Eilean (South Boisdale)	Sb
Loch nam Faoileann	F
Loch Gearraidh Mhic Iain	Gmi
Loch Grogary	G
Loch a'Mhadaidh	Mh
Loch Olaidh Meadhanach	Mlo
Loch Scarie	S
Loch an Taigh-sgoile (Schoolhouse)	Sch
Loch ? (no name)	Lo

Ireland	
Carragh lough	Ca
Cregduff Lough (Roundstone)	Cd
Fin Lough	Fi
Lough Kindrum	Ki
Leane Lough	Le
Mullaghderg Lough	Md
Lough Nafeakle	Nf
Pollacapul Lough	Pol
Renvyle Lough (Rusheenduff Lough)	Re
Lough Shennagh	Sh

Appendix 2

Water Chemistry Data

Site	pH	Conductivity	Light extinction coefficient	Alkalinity (mg/l)
Carragh Lough	7.1	63	1.60	6.71
Cregduff Lough	6.62	196	1.77	16.66
Fin Lough	6.98	54.9	1.36	5.7
Fingask Loch	8.64	370	0.67	
Glenastle Loch	7.26	272	2.35	26.35
Lake of Menteith	7.04	77	0.62	9.7
Leane Lough	7.11	143	2.35	30.16
Lindores Loch	7.45	215	3.47	98.66
Loch a' Mhadaidh	7.39	289	1.77	
Loch a'Bhada Dharaich	6.97	55	1.68	8.21
Loch Altabrug	7.97	447	2.17	25.61
Loch an Eilean (Drimsdale)	7.49	235	1.96	
Loch an Eilean (South |Boisdale)	7.98	291	2.64	17.55
Loch an t-Sagairt	6.87	163	3.05	
Loch Ballyhaugh	7.36	284	2.85	
Loch Bun an Ligidh	6.84	97	6.24	8.61
Loch Cuilc	6.88	100	2.26	
Loch Drudibeag	7.05	142	1.78	12.56
Loch Eaval	8.2	345	1.12	69.3
Loch Eilean a' Ghille-ruaidh	7.6	305	1.83	16.76
Loch Fada	6.86	293	3.19	
Loch Gearraidh Mhic Iain	8.25	305	1.96	
Loch Gorm	7.28	199	2.04	26.46
Loch Grogary	7.7	265	0.94	
Loch Kindar	7.49	160	2.12	
Loch Monzievaird	7.85	158	4.32	72.65
Loch na Cuithe Moire	7.3	183	4.07	19.15
Loch nam Faoileann	7.56	197	0.78	31.86
Loch nan Capull	7.2	143	7.33	15.9
Loch nan Cnamh	7.84	316	1.65	32.4
Loch nan Gad	8.26	145	4.61	19.51
Loch no name	7.5	405	1.91	24.51
Loch of Butterstone	8.3	139	4.06	
Loch of Clunie	7.95	198	1.33	
Loch of Craiglush	7.54	127.4	2.98	16
Loch of Drumellie	9.01	214	1.46	
Loch of Lowes	7.54	126	1.76	23.45
Loch Olaidh Meadhanach	8.17	398	1.28	40.95
Loch Poit na h-I	7.2	269	3.22	
Loch Scarie	7.14	365	0.54	69.71
Lough Kindrum	7.85	311	1.36	65.7
Lough Nafeakle	6.46	116	4.40	4.91
Lough Shennagh	7.87	239	4.40	
Monk Myre	8.79	400	1.83	
Mullaghderg Lough	7.42	281	1.91	50.86
North Loch Duidibeag	7.3	134	3.30	30
Loch Lossit	7.54	370	0.82	52.76
Pollacapul Loch	7.01	77	1.76	9.51
Renvyle Lough	7.43	235	1.46	23
Schoolhouse Loch	7.72	369	1.29	
Tangy Loch	8.78	208	5.19	41.5
White Loch	7.96	410	0.91	

Site	Ca (mg/l)	Fe (mg/l)	Mg (mg/l)	N-NH3 (mg/l)	N-TON (mg/l)
Carragh Lough	2.06	0	1.4	<0.03	<0.01
Cregduff Lough	7.96	0	3.6	<0.03	<0.01
Fin Lough	1.32	0	1.54	<0.03	<0.01
Fingask Loch					
Glenastle Loch	8.84	0	5.2	0.08	<0.01
Lake of Menteith	8.26	0	1.34	<0.03	<0.01
Leane Lough	11.42	0	2.08	<0.03	0.88
Lindores Loch	24.72	0	12	0.28	<0.01
Loch a' Mhadaidh					
Loch a'Bhada Dharaich	3.44	0	1.82	0.07	<0.01
Loch Altabrug	11.8	1	7.2	<0.03	<0.01
Loch an Eilean (Drimsdale)					
Loch an Eilean (South |Boisdale)	9.68	2	6.4	<0.03	<0.01
Loch an t-Sagairt					
Loch Ballyhaugh					
Loch Bun an Ligidh	3.88	0	3.2	<0.03	<0.01
Loch Cuilc					
Loch Drudibeag	5.72	0	4.4	0.04	<0.01
Loch Eaval	33.4	0	6.2	<0.03	<0.01
Loch Eilean a' Ghille-ruaidh	6.62	0	4.4	0.06	<0.01
Loch Fada					
Loch Gearraidh Mhic Iain					
Loch Gorm	10.14	0	5.2	<0.03	<0.01
Loch Grogary				0.036	<0.01
Loch Kindar					
Loch Monzievaird	21.7	0	8.8	<0.03	<0.01
Loch na Cuithe Moire	7.98	1	5.6	<0.03	<0.01
Loch nam Faoileann	13.12	0	4.8	<0.03	<0.01
Loch nan Capull	7.34	1	6	<0.03	<0.01
Loch nan Cnamh	13.14	0	4.8	0.05	<0.01
Loch nan Gad	8.72	0	3.2	<0.03	<0.01
Loch no name	9.5	2	4.4	0.06	<0.01
Loch of Butterstone				0.1	0.11
Loch of Clunie				0.056	1.369
Loch of Craiglush	7.48	0	2.8	<0.03	<0.01
Loch of Drumellie				0.116333	2.64666
Loch of Lowes	8.28	0	2.8	0.08	<0.01
Loch Olaidh Meadhanach	15.86	1	5.2	<0.03	<0.01
Loch Poit na h-I					
Loch Scarie	25.8	0	8.8	<0.03	<0.01
Lough Kindrum	22.4	<0.03	7.2	0.05	<0.01
Lough Nafeakle	2.02	0	2.12	0.04	<0.01
Lough Shennagh	12.32	0	4.4	0.1	0.03
Monk Myre					
Mullaghderg Lough	19.56	0	5.2	0.1	<0.01
North Loch Duidibeag	12.24	0	5.2	<0.03	<0.01
Loch Lossit	17.42	<0.03	6.4	<0.03	<0.01
Pollacapul Loch	3.58	0	1.34	0.04	<0.01
Renvyle Lough	10.46	1	5.2	0.09	<0.01
Schoolhouse Loch					
Tangy Loch	14.22	0	6.4	<0.03	<0.01
White Loch					

Site	P-DRP(PO4) (mg/l)	P-TP (mg/l)	K (mg/l)	Ortho-phosphate (mg/l)	N02-N (mg/l)
Carragh Lough	<0.001	0.021	0.46		
Cregduff Lough	<0.001	0.004	0.94		
Fin Lough	<0.001	0.012	0.3		
Fingask Loch					
Glenastle Loch	<0.001	0.011	0.7		
Lake of Menteith	<0.001	0.013	0.54	0.068	0.0055
Leane Lough	0.045	0.056	1.72		
Lindores Loch	0.074	0.16	2		
Loch a' Mhadaidh					
Loch a'Bhada Dharaich	<0.001	0.009	0.74		
Loch Altabrug	<0.001	0.004	2.02		
Loch an Eilean (Drimsdale)					
Loch an Eilean (South |Boisdale)	0.004	0.014	1.26		
Loch an t-Sagairt					
Loch Ballyhaugh					
Loch Bun an Ligidh	0.004	0.009	0.86	<0.01	0.016
Loch Cuilc					
Loch Drudibeag	0.005	0.008	1.32	<0.01	0.015
Loch Eaval	<0.001	<0.001	2.14		
Loch Eilean a' Ghille-ruaidh	<0.001	<0.001	0.94		
Loch Fada					
Loch Gearraidh Mhic Iain					
Loch Gorm	<0.001	0.015	1.54		
Loch Grogary	<0.001		1.2	<0.01	0.011
Loch Kindar					
Loch Monzievaird	<0.001	0.014	1.52		
Loch na Cuithe Moire	<0.001	<0.001	2.02	<0.01	0.016
Loch nam Faoileann	<0.001	<0.001	1.48	<0.01	0.017
Loch nan Capull	<0.001	0.01	0.64	<0.01	0.034
Loch nan Cnamh	<0.001	0.004	1.9		
Loch nan Gad	<0.001	0.02	0.16	0.015	0.008
Loch no name	0.003	0.017	0.56		
Loch of Butterstone	<0.001		1.1	<0.01	0.007
Loch of Clunie	<0.001		1.2	<0.01	0.015
Loch of Craiglush	<0.001	0.013	0.52		
Loch of Drumellie	0.023		1.36667	0.023	0.015
Loch of Lowes	<0.001	0.026	1.1		
Loch Olaidh Meadhanach	<0.001	<0.001	1.44		
Loch Poit na h-I					
Loch Scarie	<0.001	<0.001	1.78	<0.01	<0.01
Lough Kindrum	<0.001	0.01	2.74		
Lough Nafeakle	<0.001	0.012	0.54		
Lough Shennagh	<0.001	0.006	2.82		
Monk Myre					
Mullaghderg Lough	<0.001	0.013	1.22		
North Loch Duidibeag	<0.001	0.003	0.74	<0.01	0.016
Loch Lossit	<0.001	0.011	0.72		
Pollacapul Loch	<0.001	0.006	0.46		
Renvyle Lough	<0.001	0.01	1.66		
Schoolhouse Loch					
Tangy Loch	<0.001	0.017	0.3	<0.01	<0.01
White Loch					

Sediment chemistry

Site	Available P (mg/l)	Available K (mg/l)	Extractable Mg (mg/l)
Carragh Lough	2.9		
Cregduff Lough	2.8		
Fin Lough	0.9		
Fingask Loch	84.4	104	302
Glenastle Loch	1.8	37.5	66.8
Lake of Menteith	2.6	45.7	16.3
Leane Lough	5.3		
Lindores Loch	24.1		
Loch a' Mhadaidh	1	25.9	141
Loch a'Bhada Dharaich	17.3		
Loch Altabrug	1.1	8	87
Loch an Eilean (Drimsdale)	1.3	4.8	28.7
Loch an Eilean (South |Boisdale)	2.8	9.5	66.4
Loch an t-Sagairt	3	50.4	246
Loch Ballyhaugh	2.7	16.9	141
Loch Bun an Ligidh	0.3	49	243
Loch Cuilc			
Loch Drudibeag	0.5	136	388
Loch Eaval	1.9	74.5	151
Loch Eilean a' Ghille-ruaidh	1	87.1	308
Loch Fada	1.6	4	211
Loch Gearraidh Mhic Iain	1.1	35.1	152
Loch Gorm	1.2	4	17.3
Loch Grogary	1.1	18.7	48.3
Loch Kindar			
Loch Monzievaird	21.8		
Loch na Cuithe Moire	1.2	32.5	77.6
Loch nam Faoileann	1.3	15.7	50.9
Loch nan Capull	0.3	166	344
Loch nan Cnamh	3.7	104	213
Loch nan Gad	3.1		
Loch no name	3.4	67.3	211
Loch of Butterstone	6.1	29	75
Loch of Clunie	3.6	95.5	110
Loch of Craiglush	2.3	29	65.1
Loch of Drumellie	21.8	89.2	103
Loch of Lowes	9.4	15.9	21.2
Loch Olaidh Meadhanach	3.6	38.1	47.1
Loch Poit na h-I	2.2	38.2	92.4
Loch Scarie	2.6	49.7	53.7
Lough Kindrum	3.7		
Lough Nafeakle	2.9		
Lough Shennagh	5.7		
Monk Myre	11.5	28.3	54.1
Mullaghderg Lough	4.2		
North Loch Duidibeag	0.4	37.5	58.3
Loch Lossit	2.8	21.1	85.1
Pollacapul Loch	3.9		
Renvyle Lough	7.2		
Schoolhouse Loch	1.7	28	340
Tangy Loch	5.3		
White Loch	72.9	65	189

Site	Extractable Ca (mg/l)	Total Fe (mg/kg)	Total N (mg/kg)	Extractable Na (mg/l)
Carragh Lough	383	9420	360	
Cregduff Lough	4370	168000	9700	
Fin Lough	145	20600	320	
Fingask Loch	1.8	105	4980	74.9
Glenastle Loch	809	578	1880	53.9
Lake of Menteith	319	139	670	13
Leane Lough	331000	2710	830	
Lindores Loch	3760	11300	3320	
Loch a' Mhadaidh	1230	1140	6220	135
Loch a'Bhada Dharaich	5720	22000	16900	
Loch Altabrug	523	534	2230	52.4
Loch an Eilean (Drimsdale)	290	126	350	21.1
Loch an Eilean (South |Boisdale)	544	254	670	36.3
Loch an t-Sagairt	1180	1250	14000	205
Loch Ballyhaugh	1130	679	3320	81.7
Loch Bun an Ligidh	1520	7780	38100	239
Loch Cuilc				
Loch Drudibeag	1930	11900	89200	
Loch Eaval	1610	1630	11400	145
Loch Eilean a' Ghille-ruaidh	1800	1510	10200	257
Loch Fada	816	516	15100	74.1
Loch Gearraidh Mhic Iain	896	184	1830	101
Loch Gorm	198	94.4	240	17.4
Loch Grogary	513	570	14600	35.6
Loch Kindar				
Loch Monzievaird	3140	11200	1540	
Loch na Cuithe Moire	*585*	2870	20700	72
Loch nam Faoileann	482	890	*21500*	*34.2*
Loch nan Capull	1710	8680	142000	
Loch nan Cnamh	1550	2530	13600	239
Loch nan Gad	1830	8930	490	
Loch no name	1440	3950	11700	300
Loch of Butterstone	885	904	2120	23.4
Loch of Clunie	1440	7850	40800	40
Loch of Craiglush	642	424	6140	24.8
Loch of Drumellie	805	216	1110	27.6
Loch of Lowes	253	121	350	8.3
Loch Olaidh Meadhanach	872	183	1040	46.4
Loch Poit na h-I	600	190	590	41.6
Loch Scarie	908	860	27100	39.7
Lough Kindrum	1530	3760	1200	
Lough Nafeakle	5250	27600	6220	
Lough Shennagh	1420	4870	570	
Monk Myre	4920	158	600	20.5
Mullaghderg Lough	1440	7370	510	
North Loch Duidibeag	467	1290	16400	53.6
Loch Lossit	875	741	2000	26.4
Pollacapul Loch	2560	21400	830	
Renvyle Lough	950	9180	290	
Schoolhouse Loch	1790	786	11700	175
Tangy Loch	969	14500	1470	
White Loch	25000	123	11400	72.5

Species data site codes as in appendix 1

	A	Ats	Ba	Bal	Bh	But	Ca	Cl	Cm	Cn	Cr	D	E	Ed	Egr	F	Fa	Fi	Fin	G	Gl	Gmi	Go	K	Ki	L
Apium inundatum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baldellia ranunculoides	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Callitriche hamulata	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Callitriche hermaphroditica	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1
Callitriche stagnalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Ceratophyllum demersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara spp.	0	0	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	0	1	0
Elatine hexandra	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0
Eleocharis acicularis	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Eleocharis palustris	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Eleogiton fluitans	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Elodea canadensis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
Elodea nutallii	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Eriocaulon aquaticum	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Fontinalis antipyritica	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1
Hydrilla verticillata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoetes lacustris	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	0	1	0	0	1	1	1	1	1	0
Isoetes setacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juncus bulbosus	0	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
Junger mannia	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Littorella uniflora	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1
Lobellia dortmanna	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	1	1	1	1	0
Menyanthes trifoliata	0	1	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0
Myriophyllum alterniflorum	0	1	1	0	0	1	0	1	1	1	0	0	1	1	0	1	0	1	0	1	1	1	1	1	0	1

	Le	Li	Lo	Lom	Lw	M	Md	Mh	Mlo	Mm	Mo	Nc	Nd	Nf	Ng	Po	Pol	Re	Cd	S	Sb	Sch	Sh	T	W
Apium inundatum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Baldellia ranunculoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Callitriche hamulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Callitriche hermaphroditica	1	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1
Callitriche stagnalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratophyllum demersum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara spp.	1	1	1	0	0	1	0	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1
Elatine hexandra	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0
Eleocharis acicularis	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Eleocharis palustris	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0
Eleogiton fluitans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elodea canadensis	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	0	1	1
Elodea nutallii	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Eriocaulon aquaticum	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Fontinalis antipyritica	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0
Hydrilla verticillata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Isoetes lacustris	1	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	0
Isoetes setacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juncus bulbosus	0	0	1	1	0	0	1	1	1	0	0	1	1	1	0	1	1	0	1	0	0	1	1	0	0
Junger mannia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lemna minor	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Littorella uniflora	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1
Lobellia dortmanna	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	0	0	1	0	0	0
Menyanthes trifoliata	0	0	1	0	0	1	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0
Myriophyllum alterniflorum	1	0	0	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1

	A	Ats	Ba	Bal	Bh	But	Ca	Cl	Cm	Cn	Cr	D	E	Ed	Egr	F	Fa	Fi	Fin	G	Gl	Gmi	Go	K	Ki	L
Myriophyllum spicatum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Najas flexilis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1
Nitella spp.	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
Nuphar lutea	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Nymphae alba	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
Persicaria amphibia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton alpinus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Potamogeton berchtoldi	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0
Potamogeton crispus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Potamogeton epihydrus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Potamogeton gramineus	0	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1
Potamogeton lucens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton natans	0	1	1	1	1	0	1	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0
Potamogeton obtusifolius	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton pectinatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Potamogeton perfoliatus	0	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Potamogeton polygonifolius	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Potamogeton praelongus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Potamogeton pusillus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton rutilus	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
Potamogeton x nitens	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
Potamogeton x zizii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus aquatilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0
Ranunculus flamula	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparganium angustifolium	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	0	0
Sparganium emersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparganium erectum	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subularia aquatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utricularia vulgaris	0	1	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

	Le	Li	Lo	Lom	Lw	M	Md	Mh	Mlo	Mm	Mo	Nc	Nd	Nf	Ng	Po	Pol	Re	Cd	S	Sb	Sch	Sh	T	W
Myriophyllum spicatum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Najas flexilis	1	0	1	1	1	0	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0
Nitella spp.	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0
Nuphar lutea	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Nymphae alba	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0
Persicaria amphibia	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Potamogeton alpinus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton berchtoldi	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1
Potamogeton crispus	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Potamogeton epihydrus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton gramineus	0	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0
Potamogeton lucens	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Potamogeton natans	0	0	1	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	0	0	1	0	0	0
Potamogeton obtusifolius	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton pectinatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
Potamogeton perfoliatus	1	0	1	1	1	1	0	1	1	0	0	0	1	0	1	1	0	1	0	0	1	1	1	1	1
Potamogeton polygonifolius	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton praelongus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Potamogeton pusillus	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Potamogeton rutilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Potamogeton x nitens	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Potamogeton x zizii	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ranunculus aquatilis	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Ranunculus flamula	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0
Sparganium angustifolium	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1	1	0	0
Sparganium emersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparganium erectum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subularia aquatica	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Utricularia vulgaris	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0